VOL. XCIV, NO. 879 NOVEMBER-DECEMBER, 1960

THE AMERICAN NATURALIST

A BI-MONTHLY JOURNAL

Devoted to the Advancement and Correlation of the Biological Sciences

CONTENTS

Date of Issue: November 30, 1960

Published for
THE AMERICAN SOCIETY OF NATURALISTS
at
Arizona State University

THE AMERICAN NATURALIST

Established 1867

Edited in the interest of The American Society of Naturalists

Vernon Bryson, *Managing Editor*

THE AMERICAN NATURALIST is a bimonthly journal devoted to furthering the objectives of The American Society of Naturalists, which are the "discussion, advancement and diffusion of knowledge concerning the broader biological problems, including organic evolution, thus serving to correlate the various biological sciences into a common philosophy of biology." It will publish those general addresses, essays and papers presented at the symposia of biological societies which contribute to the above purpose; research papers and reviews in which theoretical interpretation and synthesis are predominant; and brief discussion, criticism and comment on material published in this journal and elsewhere. "Letters to the Editors" containing new information or comment will be dated when received and published in the next issue. They should not exceed two printed pages.

Manuscripts and editorial correspondence should be addressed to the Editor, Institute of Microbiology, Rutgers – The State University, New Brunswick, New Jersey.

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Subscription, $8.00 a year, postpaid in the United States, $8.50 in Canada, $8.75 elsewhere. Special rates for students; apply to the publisher. Single copies may be purchased for $1.35 for issues of the current year and $2.00 for issues after 1959; $3.00 for earlier issues.

THE AMERICAN NATURALIST
Arizona State University
Annex 15
Tempe, Arizona

Second-class postage paid at Lancaster, Pa., and at additional mailing offices.

THE
AMERICAN NATURALIST

Vol. XCIV The American Naturalist No. 879

VARIATIONS IN THE TRANSMISSION RATIOS OF ALLELES THROUGH EGG AND SPERM IN *MUS MUSCULUS**

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The most striking fact about Mendel's principle of segregation is the universality of its application. It is co-extensive with genes, and genes as we know are co-extensive with life. In a sense, the whole structure of genetics arose from this principle; and we who ply this trade have had our ways of thinking so conditioned by it that when there are rumors that an exception to it has been found, we feel the earth quaking beneath us. These rumors usually turn out to be groundless but they generate this salutary effect: that in the process of finding out that it is not the principle itself that is at fault we are led to discover hitherto unsuspected ways in which genes affect living processes. The shock is thus attributable to the discovery that our knowledge is faulty and not to a defect in the order in nature on which we had come to depend.

The case I am going to describe this morning has run through a part of this gamut. When some 20 years ago it became apparent that male mice were transmitting certain genes in ratios quite other than those called for by the normal operation of the segregation mechanism it could be regarded as a laboratory curiosity or anomaly in a few animals with some obscure meiotic irregularity. These mice were pretty sad and meek figures anyway, having no tails and a variety of other abnormalities connected with this condition which would prevent them from ever cutting much of a figure in nature. They did provide good material for those interested in the ways in which genes control developmental processes, especially in early embryos, but experimental embryologists didn't like them because of the inaccessibility of the embryos, and cytologists didn't like them because their chromosomes were small and not well differentiated. And when it developed that the same genes with abnormal male segregation ratios were both lethal and common in populations of wild house mice, they made a good bid for low regard by population geneticists as well. For here were animals which refused to distribute their genes in the ways called for by the bionomial calculus on which

*Presented at the symposium on Mechanisms of Abnormal Genetic Ratios at the meeting of the Genetics Society of America at Pennsylvania State University, September 2, 1959.

all gene frequency and equilibrium estimates in populations had been based. It was a horrid thought that if other animals or plants had learned to do this trick, but had skillfully concealed it from the investigators, then the results of hundreds of experiments in population cages or breeding plots might have a meaning other than that attributed to them. Of course no one had very serious doubts about this: Mendel, Hardy, Weinberg, Wright and Dobzhansky probably could not all be wrong at the same time.

But it was sufficiently disturbing to cause a group of us at Columbia to devote several years to a study of these abnormal ratios which are characteristic of alleles at one locus as found both in laboratory stocks and in heterozygotes taken from wild populations.

THE TEST SYSTEM

The opportunity for studying aberrant transmission ratios was given by the occurrence of allelic mutants at locus T in the ninth linkage group (cf. Dunn, 1956, for review). The genotypes and phenotypes involved are:

T/T — lethal at 10¾ days post-fertilization.
$T/+$ — viable with short-tail = Brachyury.
$+/t^n$ — (t^n is anyone of a series of recessives at this locus) viable with normal tail.
T/t^n — viable; tailless.
t^n/t^n — some t-alleles are lethal before birth, others are viable with normal tails.

No recombinants between T and t^n have been found so that $T/t^n \times T/t^n$ matings constitute balanced systems. New alleles are detected when normal-tailed exceptions are found among the usually tailless offspring of balanced lethal matings $T/t^n \times T/t^n$. The exceptions when fertile prove to be t^n/t^x, t^x being an allele different from and complementary to the lethal t^n allele of the parent line. Some of the new alleles are lethal, others are viable.

THE ABNORMAL RATIOS

The problem was first disclosed by comparing the results of reciprocal test matings of tailless (Tt) × wild type (+/+) animals (table 1). When the tailless animal was a male, a variety of ratios of normal ($+/t$) to Brachy ($T/+$) was found depending on the t-allele involved (table 1). The original alleles t^0 and t^1 gave high ratios (.73 and .87); new alleles derived as exceptions from these gave high (.90), normal (.5), and low (.45-.36) ratios (table 2). Males with the same abnormal ratio allele showed significant heterogeneity in the distribution of the two classes of offspring in successive litters, a phenomenon we have referred to as clustering.

When the females were tailless (having the same t-alleles as the males) the results were quite different. Females of all kinds gave normal ratios and there was no significant heterogeneity in the ratios from females with the same allele or among the females with different alleles.

TABLE 1

Transmission ratios of males T/t^n mated with +/+ females. Females $T/t^n \times +/+$ males shown as controls.

	Offspring at birth			
	Normal (+/t)	Brachy (+/T)	Total	Proportion of *t*
Males tested				
12 T/t^0	805	299	1104	.729
10 T/t^1	538	81	619	.869
6 T/t^3 *	268	323	591	.453
8 T/t^4	472	708	1180	.400
3 T/t^7 *	207	212	419	.494
3 T/t^8 *	199	205	404	.492
5 T/t^9	255	306	561	.453
3 T/t^{12}	291	32	323	.901
Females tested				
17 T/t^1	61	61	122	.500
23 T/t^3	72	74	146	.494
47 T/t^4	146	145	291	.501
8 T/t^7	22	21	43	.51
19 T/t^8	54	48	102	.539
33 T/t^9	91	100	191	.477

* Viable alleles.

The same contrast between reciprocal crosses appeared when we compared tailless males with *t*-alleles derived each from a single heterozygote ($+/t^w$) taken from a different wild population and with a series of alleles each derived from an exception from one of the balanced lines so established. Here the original alleles taken from the wild all showed high ratios (15 above .90; one of .89; table 3). Females with these alleles all gave normal ratios with no significant heterogeneity among them. The alleles derived from the above ranged from .99 to .38 with several giving normal ratios (table 4). Again there was significant heterogeneity among males

TABLE 2

Transmission ratios of tailless (T/t^n) males carrying alleles derived from exceptions in balanced lethal lines T/t^1 and T/t^{12}.

Males tested	Offspring			Proportion of *t*
	Normal (+/t)	Brachy (+/T)	Total	
10 T/t^{13}	207	382	589	.351
7 T/t^{20}	296	57	353	.839
3 T/t^{22}	96	99	195	.492
3 T/t^{24}	41	40	81	.506
4 T/t^{25}	228	229	457	.500

t^{13}, t^{22}, t^{24}, t^{25} are viable alleles; t^{20} is a lethal.

TABLE 3

Transmission ratios of males, heterozygous for *t*-alleles, each derived from a different wild population. Five males of each allelic type were tested by mating with wild-type +/+ females.

Males tested	Offspring classified at birth			Proportion of t^w
	Normal ($+/t^w$)	Brachy ($+/T$)	Total	
1	2	3	4	5
$T/t^w 1$	329	38	363	.895
$T/t^w 2$•	325	17	342	.950
$T/t^w 3$	355	3	358	.992
$T/t^w 4$	187	5	192	.974
$T/t^w 5$	479	30	509	.941
$T/t^w 6$	230	4	234	.983
$T/t^w 7$•	214	16	230	.930
$T/t^w 8$•	178	25	203	.876
$T/t^w 10$	240	7	247	.972
$T/t^w 11$	518	19	537	.965
$T/t^w 12$	412	19	431	.956
$T/t^w 13$	502	11	513	.979
$T/t^w 14$	392	21	413	.949
$T/t^w 15$	415	41	456	.922
$T/t^w 16$	107	6	113	.947
$T/t^w 17$	336	1	337	.998
All ♂♂	5215	263	5478	.952
All ♀♀ T/t^w	199	188	387	.517

•Viable alleles.

with the same allele and among litters from the same male whenever the allele gave abnormal ratios but not when the allele gave normal ratios.

It was evident that the abnormal ratios were peculiar to males and that heterogeneity in segregation ratios both within and between individuals was associated only with the abnormal ratios.

The two original alleles in the laboratory stocks and four of the different alleles derived from exceptions from these were lethals. All of these gave

TABLE 4

Transmission ratios of tailless males (T/t^w) carrying alleles derived from exceptions in balanced lethal lines from wild populations

Males tested	Offspring			Proportion of t
	Normal ($+/t^w$)	Brachy ($+/T$)	Total	
12 $T/t^w 18$	426	301	727	.586
7 $T/t^w 19$•	126	208	334	.377
6 $T/t^w 20$	227	2	229	.991
7 $T/t^w 21$	216	2	218	.991
3 $T/t^w 22$•	28	32	60	.467
4 $T/t^w 24$•	59	58	117	.504
4 $T/t^w 25$•	81	76	157	.516
5 $T/t^w 27$•	132	128	260	.508

• Viable alleles.

abnormal ratios from males, four high, two low; of seven viable alleles derived from these, five gave normal ratios, two gave low ratios. Amongst the original and derived wild alleles 16 were lethals, all with high ratios, eight were viables, three with high, four with normal, one with a low ratio. Thus of 22 lethals tested all gave abnormal ratios (20 high, two low) while of 15 viable alleles, three (all taken directly from the wild) gave high ratios, nine gave normal ratios, and three low. Lethality thus always entails abnormal ratios while viable alleles may or may not have them. The relevant data are shown in table 5.

TABLE 5

Distribution of male transmission ratios

	.99 .90	.89 .80	.79 .70	.69 .60	.59 .55	* .50	.49 .45	.44 .40	.39 .30	.29 .20	n
All lethals	15	3	1		1		1	1			22
All viables	2	1				9	1		2		15
Original lab		1	1								18
Original wild	15	1									
All derived	3	1			1	9	2	1	2		19

*Ratios within $\pm 2\sigma$ of .5, i.e. normal ratios.

In this table the 37 alleles studied are classified in a second way, as "original," those which were present in laboratory stocks or wild populations, or "derived"—those which originated under observation in exceptions from balanced lethal stocks. It is the latter which we referred to in early publications as "mutants" although we supposed from the frequency with which new instances of them occur ($\frac{1}{500}$ to $\frac{1}{2000}$ in the case of different "original" alleles) that they were not due to point mutations. We know now that many of them result from recombination in the neighborhood of T-locus. From the comparison in table 5 an interesting distinction occurs: all original alleles without exception have high male segregation ratios while the derived alleles have ratios scattered over the whole range from .99 to .35. The meaning of this seems clear. It is only alleles favored by high segregation ratios which fail to be eliminated by selection against the homozygous lethal effects or the male sterility of those alleles which are viable. When we test unknown populations whether from laboratory stocks or from nature, it is these alleles which we find in heterozygous form $+/t$, usually in high frequency. The conditions which permit these to persist and spread and at the same time to keep their frequencies within certain limits present one of the most interesting evolutionary aspects of variable segregation ratios which I shall discuss later.

Just now I want to raise the question of chief relevance to this symposium: what is the mechanism responsible for the marked departures from normal Mendelian ratios of *t*-alleles when they occur in male heterozygotes? As we have seen, female heterozygotes show perfectly normal segregation, so we have to look for something unusual either in the production or the

functioning of the two types of sperm in heterozygotes. Our first guess was that spermatogenesis was abnormal in such heterozygotes, as in the two cases most similar to this one—"sex ratio" and segregation—distorter in Drosophila. But we have never found any evidence of this.

Two sets of observations point to a different kind of interpretation, namely to effects of a *t*-allele on the behavior of the sperm which carries it by which its chance of fertilizing an egg differs from that of a non-*t* sperm. Clusters of offspring with one allele or the other in great excess are found in individual litters from heterozygous males giving abnormal ratios, and only from them. Bryson (1944) suggested that if physiological differences exist between *t* and non-*t* sperm, then clusters of fertilizations by one or the other could arise under chance conditions of mating. The other set of observations are those of Braden (1958) that an important factor is the length of time intervening between copulation and penetration of the sperm into the egg. Braden's results provided the first experimental evidence by which the cluster phenomenon could be interpreted. Now an hypothesis consistent with many of the facts of abnormal transmission can be devised, although the facts now known do not provide a full test of the hypothesis. I shall first describe Braden's experiment and then apply his interpretation to our breeding results.

In earlier work (Braden, 1957) it had been observed that under normal conditions mating occurs (in the strain of females used in the experiments) about five hours before ovulation and sperm penetration of the egg occurs about eight hours after mating. By withholding males from access to females until after ovulation had occurred, the interval between mating and sperm penetration was reduced to about one hour. His results (with one unexplained exception) are consistent in showing that time of mating influences the ratio of *t* and *T* sperm which effect fertilization. When mating occurs at the normal time the sperm with *t* are superior, resulting in transmission ratios similar to those shown for these alleles in our tests (table 1) in which females were continuously exposed to *T/t* males. When mating is artificially delayed until after the time of ovulation, the superiority of *t* sperm is reduced. In terms of transmission ratios, a *t*-ratio of .76 is reduced to .53 (t^1); one of .82 to .60 (t^0) and in the case of two T/t^{12} males which showed the effect from .92 to .83. We have confirmed this result for one allele t^1 which in late-mated females gave transmission ratios close to 50 per cent. This suggests that the longer interval between mating and egg penetration favors the sperm with *t*. Braden confines himself to the conclusion that "the mechanism of the segregation ratio abnormality in mice carrying a *t*-allele has a physiological rather than a cytological basis and that its time of action is between ejaculation and fertilization." The essential mechanism on this interpretation is thus quite different than in the other cases reviewed this morning and inheres in the influence exerted on the behavior of a sperm by a gene contained within it. The implications of such an hypothesis are that the transmission ratio abnormalities of males with *t*-alleles constitute exceptions not to the principle of segregation but to the

assumption of random union of gametes in fertilization. Before such a conclusion can be accepted however we shall have to know whether conditions exist under which sperm with and without a *t*-allele associated with a high ratio have *equal* probability of fertilizing an egg. Restoration of a 50:50 ratio from a higher one has been approached; if it is regularly accomplished by late mating we can then absolve the act of segregation itself and speak in this case only of transmission ratios as I have already done in the title. We shall have to know also whether the same interpretation applies as well to alleles associated with very high and with low transmission ratios. Our results from very high ratio males are negative, but decision awaits the completion of further experiments. If departures in both directions from the normal ratio are found to have a physiological basis in sperm behavior, then an additional means of characterizing the effects of different alleles at this locus will be available. The implications would be that some of the alleles affect sperm behavior while others do not.

As things stand however it seems to me that Braden has found an essential clue to a long-standing puzzle and I am tempted to apply his hypothesis to the interpretation of some of the peculiarities in the data on transmission ratios which I presented earlier. As I pointed out, abnormal ratios seem always to be associated with heterogeneity amongst males of the same *t*-genotype in the ratios they produce. This heterogeneity is in turn associated with non-random distribution of frequencies of offspring with and without *t* within the litters sired by the same male.

To illustrate this the record of a male T/t^0 tested by normal (+/+) females may be cited. He produced 137 normal ($+/t^0$) and 17 Brachy ($T/+$) offspring in 18 litters. One litter containing one normal and seven Brachys contributed 47 out of a total chi-squared of 60 computed for all litters. The probability that the frequencies were distributed at random among the litters was less than .001. Such "clusters" occur within the litters of many males showing abnormal ratios. The occurrence of such non-random events will then introduce heterogeneity amongst different males subject to them. Such clusters, on Braden's hypothesis could be due to delayed mating relative to ovulation and could be due to assignment to a pen in which a male was being ratio-tested of a female who by chance had just ovulated. The oestrus state of females in such test crosses was not controlled. Since males were continuously penned with test females which were removed when pregnant and replaced by others, most matings would be expected to occur at the normal time, that is about five hours before ovulation. We are now testing experimentally the relationship between mating time, ovulation, and the occurrence of clusters.

EVOLUTIONARY ASPECTS OF ABNORMAL TRANSMISSION RATIOS

When it was found that males from wild populations heterozygous for a *t*-allele regularly transmitted this allele to about 95 per cent of their progeny, I pointed out (1953) that this by itself would confer so great an advantage on the *t*-allele that other things being equal it should spread through

any population in which it occurred and attain high frequencies. Prout (1953) and later Bruck (1956) worked out mathematical models for this newly discovered evolutionary force and showed that even with complete negative selection against homozygotes, lethal *t*-alleles should reach equilibrium values determined by the transmission ratio advantage, approaching a limit of 50 per cent gene frequency (100 per cent heterozygotes) as the transmission ratios approached their limit of one. In these models other evolutionary parameters were assumed to be those of the general Hardy-Weinberg equilibrium—infinite population size, no mutation pressure, and equal selection coefficients for +/+ and +/*t*. These results posed a paradox, first in predicting virtual fixation of a lethal (surely a good road toward race-suicide) and second in predicting gene frequencies well above those found in natural populations, which in our experience never exceed 25 to 30 per cent (50 to 60 per cent of heterozygotes). This suggested that the model assumptions concerning other parameters differed from conditions in nature, and some tests of these have now been made. Selection against heterozygotes is probably not an important force restraining the rise of gene frequencies due to transmission ratios, since two preliminary tests suggest that heterozygotes have higher adaptive values than homozygotes. There is no evidence of unusual mutation pressure from + to *t* and this could in any case hardly be a restraining force in controlling the rise in gene frequency. Recently, the effect of small effective size of breeding units has been examined in a stochastic model (Lewontin and Dunn, 1960) and it has been found that here indeed is a means of restraint through chance loss of alleles. Breeding groups where effective size is very small (eight and smaller) and transmission ratio high (.95 and over), lethal *t*-alleles even when present initially in the frequencies found in wild populations tend to be lost from some of the populations. There is a clear prediction from the model that chance alone will lead to fixation of wild type alleles in a fraction of the small breeding groups. Whether the breeding units in nature are in fact small enough to have this effect we do not yet know. This is now being studied by ecologists interested in this problem.

Another possible restraint on the spread of high ratio alleles can be considered in the light of Braden's hypothesis. One consequence of the mechanism by which he supposes the high ratio abnormality to operate would be that shortening of the interval between mating and sperm penetration should reduce the relative advantage of the high ratio *t*-alleles. We do not yet know whether the reproductive cycles of mice living under natural conditions are the same or different from those living under the laboratory conditions in which our ratio estimates were obtained. Here is another factor which must now be taken into account in drawing up a balance sheet of evolutionary forces acting on *t*-alleles. The reproductive physiology of animals in natural populations has interested ecologists for a long time. A new use or application of such knowledge to evolutionary problems can now be foreseen.

All in all, I think we can be grateful that a clever species has found ways of evading the evolutionary consequences of what, by the standard rules of gene frequency regulation, seemed like a fatal stacking of the cards against it. If we do succeed in finding out how the mouse gets away with it, we may be able to make better guesses as to how other species adjust their structures to the conditions of a hazardous world.

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Vol. XCIV, No. 879 The American Naturalist November-December, 1960

EFFECTS OF SIZE ON FECUNDITY, LONGEVITY AND VIABILITY IN POPULATIONS OF *DROSOPHILA PSEUDOOBSCURA**

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It is well known that body size of Drosophila is determined by interaction of genetic and environmental factors. The evidence for this is derived from investigations carried out mainly on *Drosophila melanogaster* to study the inheritance of wing and thorax length which are correlated with size. The work of Robertson and Reeve (1952) and their series thereafter and Tantawy (1957 and 1959) show that variation in body size of *Drosophila melanogaster* is due more to additive genetic variance rather than to environmental variations. On the other hand, Bell, Moore and Warren (1955) and Robertson (1957) found that variations in egg production are conditioned by environmental agencies more than genetic variations. Thus the environmental conditions would seem to play a greater role in determining the egg laying capacity than the body size of an individual female. Robertson and Sang (1944a, b) reported that the rate at which eggs are laid by *Drosophila melanogaster* is very sensitive to variations in the environmental conditions of both larval and adult flies. Reeve (1954) discussed the relationship between the egg production and body size, and pointed out the need for more experimental investigations of this problem.

The present experiment was designed to study the effects of size, that is wing and thorax length, in populations of *Drosophila pseudoobscura* on lifetime egg production, longevity of adult females and viability under various environmental conditions.

TECHNICAL PROCEDURE

Two cages Nos. 3 and 6 of populations of *Drosophila pseudoobscura*, homozygous for the Arrowhead (AR/AR) gene arrangement in the third chromosome, which were maintained for about two years at 25°C. and 15°C., respectively, were used as sources of material for the present investigation.

At the beginning of the experiment, cups were taken from each cage to obtain virgin females. From each cage 200 virgin females were obtained during the first twelve hours of hatching. All virgin females were measured for wing and thorax length by the method devised by Robertson and Reeve (1952).

The technical procedure used for females obtained from cage No. 3 was carried out in the following way. Each individual female was measured and

*This work has been carried out during the tenure by the senior author of the Boese Fellowship at Columbia University, New York.

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put in a separate food vial; flies with large, medium and small wing length were then selected. The first two largest females were selected, one to be kept at 25°C. and the other at 15°C. The same procedure was used for the next two largest females, and so on for the first sixty females; that is, thirty females were selected for long wing length to lay eggs at 25°C., and another thirty females of almost the same size were selected to lay eggs at 15°C. At the same time sixty virgin females of medium size and another sixty females of small size were selected; half of each group were used for laying eggs at 25°C. and the other half at 15°C.

An exactly similar procedure was used for females obtained from cage No. 6. Measurements and selection for wing length were all done during the first twenty hours after hatching. Therefore, 360 virgin females were selected for laying eggs, thirty females in each group at each of the two temperatures.

Each selected female was mated in a separate vial to three males from the same cage; each vial contained a paper spoon with usual cream-of-wheat molasses medium with a drop of Fleischmann's yeast. Every day at 9 A.M. spoons were taken out of the vials and immediately new spoons with food and yeast were inserted in the vials. Eggs were counted on each spoon under a binocular microscope. Dead females were replaced by other females of about the same size, if possible, but only during the first ten days of their age after which no replacements were made. Spoons with counted eggs of the first ten largest females and the first ten smallest females in the large and small selected groups, were placed in food bottles. Spoons with eggs produced by an individual female for every two successive days were put in one food bottle. After hatching of the adults, flies were counted and classified as to sex. Such counting was made first two days after hatching, and thereafter once every five days for two weeks.

All adult females used for laying eggs and the culturing of eggs in food bottles were treated similarly to assure the same environmental conditions under all temperatures. The grand total number of eggs counted in the present work was 384,965 eggs, for all groups under the two temperatures.

RELATION BETWEEN SIZE AND LIFETIME EGG PRODUCTION

The relationship between females with large, medium and small wing length, and the capacity for laying eggs during the lifetime at different temperatures is reported in figures 1 and 2, for 25°C. and 15°C., respectively. The results are presented as averages per day on a given week. No eggs were laid during the first two days of the adult female life at 25°C., and for the first four days at 15°C.

The results reported in figures 1 and 2 show clearly the effects of size on lifetime egg production; larger females lay more eggs than medium or small ones at both temperatures. The average daily productivity per week increases rapidly after the first week at 25°C. and somewhat more slowly at 15°C. The period of high egg production varies considerably with temperature, but is uniform for flies of different sizes. At 25°C. the highest

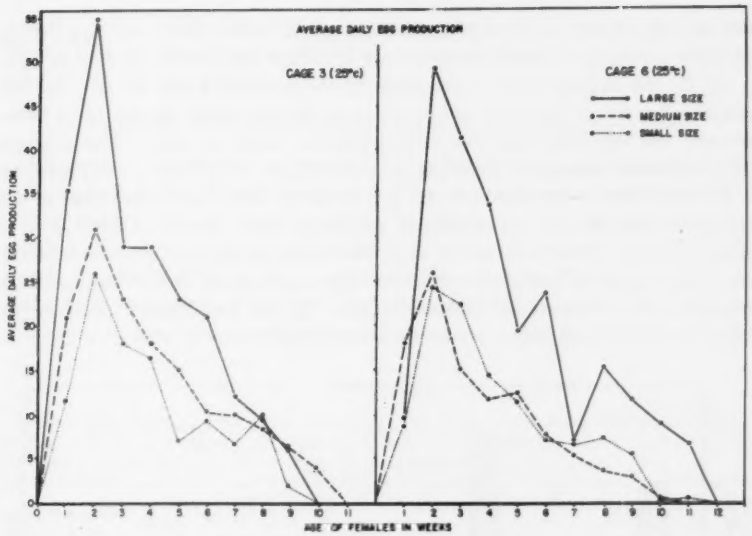

FIGURE 1. Lifetime egg production for large, medium and small selected females for wing length at 25°C. Each point represents the daily average on a given week.

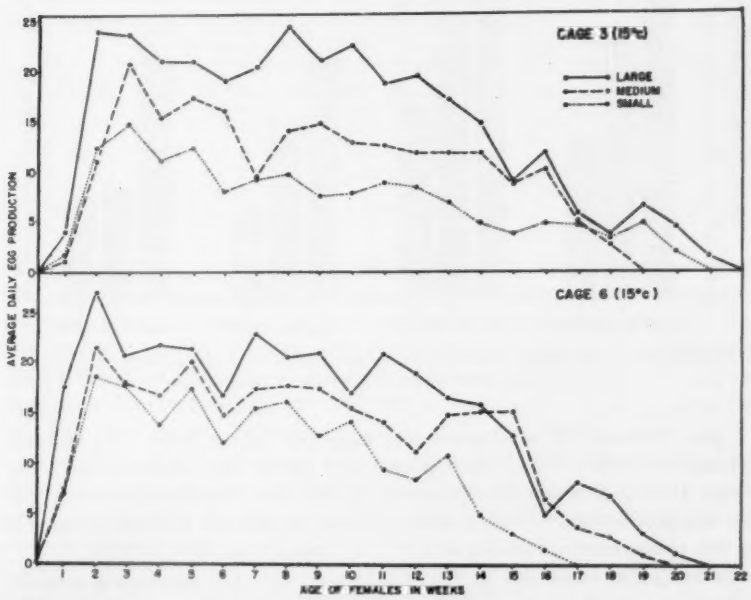

FIGURE 2. Lifetime egg production for large, medium and small selected females for wing length at 15°C. Each point represents the daily average on a given week.

peak of egg laying is during the second week, after which the egg laying declines gradually to zero, between the eleventh and twelfth weeks of age. At 15°C. the highest peak falls also on the second week of life for all groups, after which the decline in the egg production goes gradually to zero, between the twentieth and the twenty-second weeks of age. The average egg production during the peak periods under the two different temperatures is different, and much higher at 25°C. It can be also noted that most of the egg production occurs at an earlier period at 25°C. and at a later one at 15°C. Similar results were reported by Dobzhansky (1935) on unselected flies of *Drosophila pseudoobscura*, although our results differ from his with respect to the duration of active lifetime. In his experiment, the females reared at 25°C. laid eggs only as far as the sixth week of age.

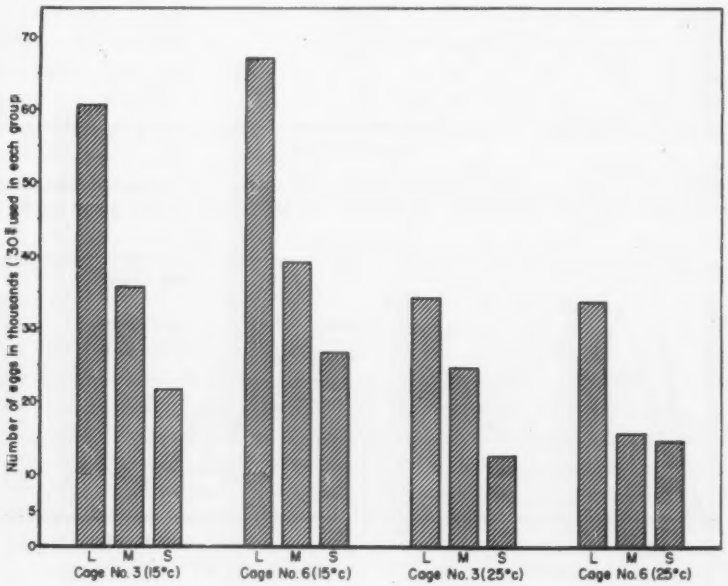

FIGURE 3. Total ovaposition rate in relation to size of the selected females for wing length at different degrees of temperature.

Figure 3 shows the total number of eggs laid by the same total numbers of females (30 females in each group) kept at the two temperatures. The results show highly significant effects of the female body size on the lifetime egg production. There is also a highly significant difference between the two temperatures; females at 15°C. laid more eggs than at 25°C.

Spiess (1958) found that in *Drosophila persimilis* the egg laying capacity of females raised under conditions of high larval density does not differ significantly from that of females raised under near optimal conditions. It is well known from the results reported by many investigators, for ex-

ample, Sang (1950) that larval crowding affects the size of the resulting flies, that is, the flies hatched from crowded cultures are smaller in size than those from optimally populated ones.

PHENOTYPIC VARIATIONS OF BODY SIZE AND EGG PRODUCTION

Phenotypic variations of the body size and the egg production are calculated as coefficients of variation, and the results are presented in table 1, which shows that the coefficients of variation for the egg production are much higher than those for body size. This indicates that the former character is affected by environmental conditions more than the latter one. Robertson and Reeve (1955) found that the relative variance of the fecundity of *Drosophila melanogaster* is about 200 times as that for body size. They

TABLE 1

Total lifetime egg production per female in relation to wing length and thorax length. Means are presented with their respective standard errors (S.E.) and coefficients of variation (C.V.). Units of measurement are of $^1/_{100}$ mm.

Cage No.	Temperature	Size	Wing length		Thorax length		Egg production	
			Mean ± S.E.	C.V.%	Mean ± S.E.	C.V.%	Mean ± S.E.	C.V.%
3	25°C.	Large	217.47 ± 1.56	2.58	104.09 ± 0.94	3.27	1131.69 ± 146.61	46.64
		Medium	198.63 ± 2.23	5.61	94.32 ± 1.32	6.99	822.32 ± 80.36	48.86
		Small	180.03 ± 3.52	6.05	83.64 ± 2.10	7.70	409.20 ± 85.23	64.57
6	25°C.	Large	232.22 ± 2.53	3.49	106.59 ± 1.06	3.10	1127.90 ± 142.09	40.31
		Medium	211.77 ± 2.51	4.39	99.59 ± 1.73	6.43	512.14 ± 108.57	78.44
		Small	182.50 ± 3.78	7.45	86.70 ± 1.47	6.11	502.23 ± 125.22	89.77
3	15°C.	Large	217.46 ± 2.50	3.91	105.10 ± 1.68	5.42	2021.00 ± 72.35	12.17
		Medium	201.84 ± 2.10	4.16	95.96 ± 1.28	5.31	1174.19 ± 132.45	45.12
		Small	173.86 ± 2.00	3.80	81.91 ± 1.88	7.57	726.73 ± 8.75	39.73
6	15°C.	Large	233.15 ± 2.61	3.69	107.84 ± 1.42	4.36	1894.73 ± 319.75	55.69
		Medium	206.47 ± 4.42	8.14	99.17 ± 2.82	10.79	974.27 ± 183.71	71.73
		Small	187.77 ± 3.88	6.82	90.45 ± 2.30	8.40	890.27 ± 230.03	85.22

also reported that inbreeding causes a decline in the egg production and the size; the latter is, however, relatively less affected than the former. The egg production may be regarded as more sensitive than the body size with respect both of environmental and genetical changes. Our results indicate that changes in environmental conditions cause changes in egg production and its variability; at 25°C. the egg production is much lower than at 15°C., the latter temperature showing less affect on the variability. A similar difference is observed between large and small flies, the egg production of the former is less variable than that of the latter. These results are in agreement with those reported by Gowen and Johnson (1946) for *Drosophila melanogaster.*

LONGEVITY AND LIFETIME EGG PRODUCTION

Table 2 shows the relationship between wing length, the longevity, and the average daily egg production. The selection differentials are measured

as differences between the wing length in the selected females and the population mean from which they were selected. The results show that the higher averages of egg production are associated with higher selection differentials for female wing length and for her longevity. In all cases larger females live longer and produce more eggs than smaller or medium ones. It could, therefore, be concluded that the metabolic activity as measured by egg production capacity is correlated with the physiological fitness as measured by female capacity to survive. These results are in agreement with those reported by Dobzhansky (1935).

Table 2 illustrates that the daily average egg production is higher at 25°C. than at 15°C., but the total lifetime egg production (figure 3) is much higher at the latter temperature than at the former. Such differences are due to the fact that adult females live longer at a lower temperature than at a higher one.

TABLE 2

Average daily egg production per an individual female in relation to selection differential (S.D.) for female wing length and longevity of adult females. Standard errors (S.E.) for means are also presented. Units of measurements are of $\frac{1}{100}$ mm.

Cage No.	Temperature	Size	S.D. ± S.E.	Average daily egg production ± S.E.	Average longevity ± S.E. (Days)
3	25°C.	Large	21.68 ± 1.58	24.51 ± 1.41	37.31 ± 5.44
		Medium	2.83 ± 2.22	14.78 ± 1.27	35.20 ± 3.50
		Small	−15.77 ± 3.68	12.57 ± 1.24	28.20 ± 5.13
6	25°C.	Large	25.19 ± 2.64	20.93 ± 2.08	36.30 ± 6.53
		Medium	4.78 ± 2.51	10.78 ± 1.22	35.18 ± 4.30
		Small	−23.66 ± 3.44	10.53 ± 1.08	31.53 ± 9.88
3	15°C.	Large	21.67 ± 2.50	15.03 ± 0.76	119.12 ± 6.50
		Medium	6.04 ± 2.10	10.79 ± 0.57	95.00 ± 6.43
		Small	−21.94 ± 2.06	7.33 ± 1.36	83.45 ± 9.91
6	15°C.	Large	26.14 ± 2.58	17.82 ± 0.81	92.91 ± 11.45
		Medium	0.53 ± 4.42	15.18 ± 0.63	80.13 ± 6.26
		Small	−19.23 ± 3.88	12.79 ± 0.64	70.00 ± 10.69

It is of interest to note from table 2 and figure 3 that the original temperature of the population may have an influence on the capacity for laying eggs. Females from cage No. 3 were reared at 25°C. However, egg production at 15°C. is different; females from cage six show a higher egg production. These results are in agreement with those reported by Tantawy and Mallah (1961) who worked on natural populations of *Drosophila melanogaster* and *D. simulans* and found that the temperature prevailing in various geographical regions affect the body size and the egg viability.

VIABILITY AND SEX-RATIO

The viability is measured as the percentage emergence of adults that hatched from a given number of eggs cultured in ordinary food bottles. Table 3 reports for ten females in each of the selected groups the total

lifetime egg production and the percentage of emergence and sex-ratio of the progeny. Size of the female parent has no significant effect on the percentage of her eggs which successfully develop to adult stage. The results do, however, indicate differences between the two temperatures; 15°C. shows a higher viability. The sex-ratios of the various groups are uniform; females slightly out-number the males.

TABLE 3

Lifetime egg production (ten females in each selected group) cultured in food bottles, with percentages of the emergence of adult flies and the sex-ratio

Cage No.	Temperature	Size	Total lifetime egg production	No. of hatched		Per cent of emergence	Sex-ratio*
				Males	Females		
3	25°C.	Large	14,712	5,002	5,293	69.97	51.41
		Small	4,967	1,642	1,800	69.29	62.29
6	25°C.	Large	11,279	3,969	4,207	72.54	51.45
		Small	6,259	2,132	2,304	70.89	51.93
3	15°C.	Large	24,257	9,704	10,171	81.91	51.16
		Small	7,944	3,213	3,405	83.30	51.15
6	15°C.	Large	20,842	8,283	8,574	80.88	50.21
		Small	9,793	4,082	4,097	83.51	50.09

*Percentage of females to males.

CONCLUSIONS

The present study, on populations of *Drosophila pseudoobscura*, shows that temperature has a highly significant effect on the egg production. Females lay more eggs at a low than at a high temperature. This agrees with the data of Alpatov (1932) on *Drosophila melanogaster* and of Dobzhansky (1935) on *Drosophila pseudoobscura*.

Robertson (1957) reported that body size of *Drosophila melanogaster* may influence egg laying capacity. Our results on the whole agree with Robertson's results, but the present work indicates a highly significant effect of the female body size on the capacity for egg laying, and on the longevity of the females. Alpatov (1929) and Sang (1950) have found that small flies which hatch in crowded cultures lay fewer eggs. However, Spiess (1958) reported different results.

The highly significant correlations found between body size and egg production may indicate that these two characters are genetically correlated. The available data do not warrant such a conclusion, but it would be interesting to investigate experimentally this problem in *Drosophila pseudoobscura*. Robertson (1957) found in his selection experiment for size in *Drosophila melanogaster* that only in one comparison, after five generations of selection, was there evidence of correlation between size and egg production; in the others the difference in the egg output of large, small and unselected groups were negligible. He concluded that a substantial change in body size by selection does not lead to parallel changes in egg produc-

tion. Our results indicate that changes in body size of *Drosophila pseudoobscura* are accompanied by changes in egg production. This statement needs to be clarified by more experimental studies on selection for large and small sizes and lifetime egg production at different generations of selection.

Results obtained clearly show that size has a great influence on egg production, on longevity of adult females, but not on egg viability. This indicates that egg viability is a character which depends entirely on parental genotype rather than their phenotypes.

SUMMARY

1. An experiment was designed to test the effects of body size in *Drosophila pseudoobscura* on lifetime egg production, longevity, and egg viability.

2. The results show that large females lay significantly more eggs than medium or small flies; small females lay the least number of eggs. Comparing the egg laying capacity at different temperatures, it is found that females at 15°C. lay more eggs than those at 25°C.

3. Larger females live longer than medium or small flies: the longevity of adult females kept at 15°C. is greater than at 25°C.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Professor Th. Dobzhansky of Columbia University, New York, for his helpful discussion during the course of the experimental work and in preparation of the manuscript. We wish to thank Dr. M. Demerec, Director of the Biological Laboratory, Cold Spring Harbor, Long Island, New York, for awarding the senior author a Summer Fellowship in 1959, which enabled him to use the laboratory and library facilities for analyzing the data and writing the manuscript.

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Note: For more statistical analyses applying the path coefficient theory to the present data, see Tantawy, 1961, Genetics, Vol. 46, No. 1 (in press).

Vol. XCIV, No. 879 The American Naturalist November–December, 1960

BITHORAX AND HETEROSIS EFFECTS ON EGG YIELD IN *DROSOPHILA MELANOGASTER*

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The following study was initiated as an attempt to analyze the basis for extremely low fecundity in a closed stock of *Drosophila melanogaster*. Breeding difficulty had been apparent ever since it was obtained in 1954 from the Carnegie Institution laboratory at Cold Spring Harbor, New York. The stock was maintained because it is homozygous for the recessive marker bithorax-34e (bx^{34e}, chromosome III, locus 58.8). According to Bridges and Brehme (1944) this mutant was discovered by Schultz in 1934; also it is stated to have "high viability," and nothing is said about fecundity.

Preliminary observations showed poor viability, the bithorax flies readily becoming stuck in the food. However, the major trouble seemed to be very low egg production. Since egg yield has been studied in relation to vigor by a number of previous investigators, we decided to use this "character" in the analysis. The contributions of the *bx* gene and its wild allele, as compared with other factors, were to be partitioned if possible.

METHODS

A plain corn-meal, molasses, brewer's yeast, agar medium was used, inoculated with live baker's yeast, in quarter-pint milk bottles. Females to be tested for egg yield were produced from cultures without over-crowding, to ensure optimal nutrition. Virgin females were obtained by etherizing within eight to ten hours after eclosion, no adult males being previously present.

It was decided to follow essentially Robertson's (1955) procedure of using females four days old and counting eggs laid for each of four successive days, since this is the period of maximum production. Except in one operation where progeny-testing was necessary, each female was mated with one or two wild-type males. Fresh food in the form of a yeast-seeded one-inch diameter drop on the waxed-cardboard bottle cap was prepared daily, and the bottles kept inverted, as in the usual procedure (Gowen and Johnson, 1946). However, instead of charcoal to darken the food, red vegetable food coloring was used, and no acetic acid was added as an incitant.

For a control normal type, several dozen wild flies were obtained in a local apple orchard about a month before starting the breeding tests. These wild flies were multiplied by mixed matings to minimize inbreeding.

*Journal Paper No. J-3920 of the Iowa Agricultural and Home Economics Experiment Station. Project No. 1380.

The breeding plan of the experiment is outlined in figure 1. In order to obtain representative populations, the F_1 was produced in ten bottles, each starting with two virgin wild females, from separate bottles, with several bithorax males. Females for egg-count tests were also chosen from these various bottles without bias.

In the backcrossing program A, two or three virgin heterozygous (*+/bx*) females were taken from previous cultures for each of ten new bottles, and mated with six to eight males from the bithorax stock. In the reciprocal backcross, B, five to ten heterozygous males were mated with 15–25 virgin females from the bithorax stock, again in ten bottles. This large number of females was necessary to ensure adequate numbers of progeny.

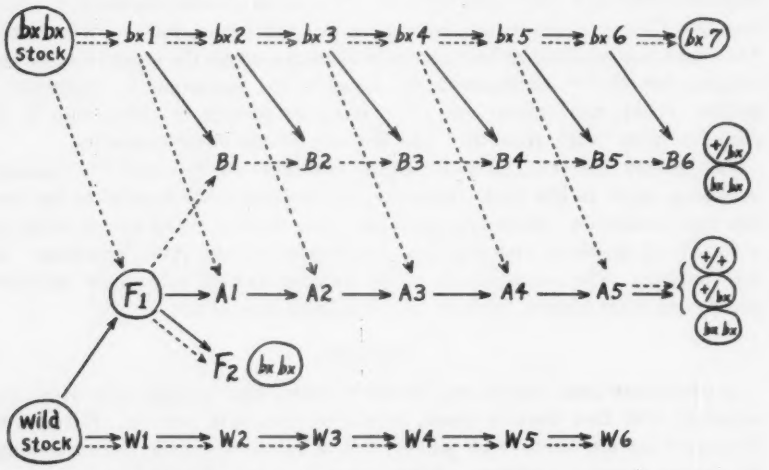

FIGURE 1. Outline of the breeding program to produce the several populations. The male origin of each group is indicated by a dashed line, female origin by a solid line. Groups sampled for egg-yielding ability are circled.

By the fifth or sixth generation of backcrossing, unless unexpected natural selection interfered, most of the chromosomal contribution from the wild ancestry of the cross should be eliminated. In the A series crossing-over should have got rid of most of the wild chromosome III except in the vicinity of the *bx* locus. In the B series, because of no crossing-over in the male heterozygotes used, an intact wild chromosome III is maintained. The final step therefore was to compare egg yields at this stage for the different segregating types.

In series B, the two classes *bx bx* and *+/bx* segregating in the sixth generation were sampled. In series A, a different plan was followed: in order to compare *+/bx* with *+/+*, heterozygotes from seven bottles of generation five were mated inter se, in 12 bottles. Of the progeny, 200 virgin wild-type females were tested for egg yield, but instead of wild-type mates

they were given several bithorax males. After counting, the eggs were put in culture bottles and the resulting progeny examined to determine whether the dam had been +/*bx* or +/+. The decision was clear except for three females (not used in the calculations) which yielded samples of less than seven progeny, all wild-type.

For statistical analysis, barren females and those not completing the four day tests have been omitted from computations (Gowen, 1952), but their frequency is noted in table 1.

TABLE 1

Statistics of egg-yield data. Symbols for the different groups of females are the same as in figure 1.

Type of ♀	Egg yield tests			Completed egg yield tests						
	Completed	Not completed	Barren	Mean	± S.E.	Median	Range	C.V. %	Variance log*	Maximum day
Wild stock	52	4	0	272	9.7	282	19-410	26	22.2	134
bx bx stock	112	19	2	23	1.4	20	2-70	63	52.1	30
F_1	50	2	0	300	11.0	312	106-445	26	11.7	118
bx bx F_2	77	5	2	103	4.1	97	48-218	35	13.6	71
bx bx B6	66	4	0	24	1.8	23	2-63	61	55.5	25
bx bx A5	85	9	1	26	1.9	26	1-73	67	118.4	37
+/*bx* B6	64	1	0	87	2.7	84	41-163	25	6.1	55
+/*bx* A5	143	8	7	110	3.4	103	20-222	37	15.8	71
+/+ A5	42			108	6.0	98	46-212	36	11.3	67

*Variance of each population based on ten $\log_e$ of mean daily production.

RESULTS

In most of the segregations no significant deviations were obtained from expected ratios. For example in the F_2 there were 357 wild: 109 *bx bx*; in series B6, a sample consisted of 96 wild: 113 *bx bx*. However in the segregation from A5, of the 200 wild-type females taken for egg counts, 185 were progeny-tested, giving a ratio of 143 +/*bx*: 42 +/+. The probability of a chance deviation from 2 : 1 of this magnitude is about .002. Even if all the 15 untested females had been +/+, the deviation would be significant at the .01 level. The presence of a recessive lethal in some of the wild chromosomes III is therefore indicated.

Females of the bithorax parent stock were sampled twice for egg yield, six generations intervening. The mean of the first test was 22.73, and of the second, 23.56. Since the stock had not changed, the two tests are combined (table 1). The means and ranges for the *bx bx* flies in both backcross series were essentially the same as that of the parent stock; the medians however indicate a very slight residual increase.

The distribution of the parental wild stock was tested for normality by the Snedecor method and found not to deviate significantly. The curve for the F_1 is more flattened. Although the F_1 exceeded the wild parent stock in four-day yield mean and range, the highest single-day record was from a wild-stock fly. The average daily egg yields for the four days were not

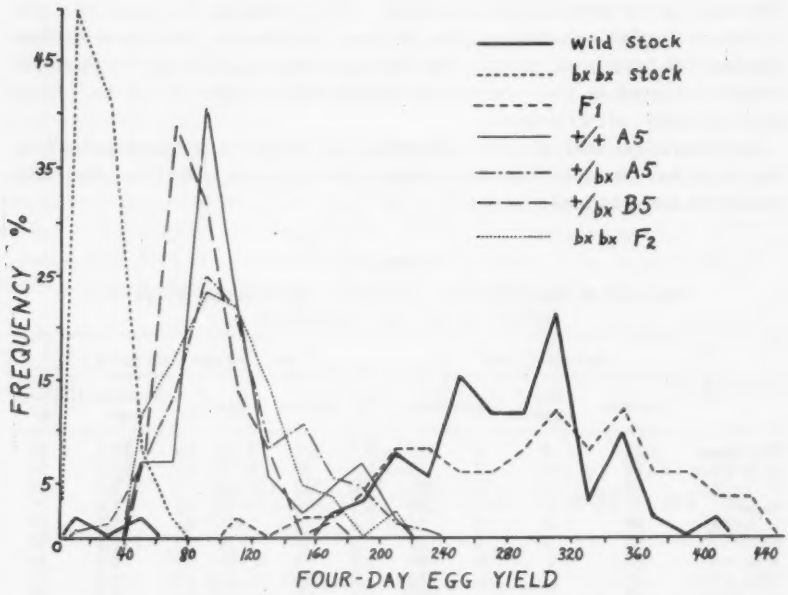

FIGURE 2. Egg yield frequency distributions for seven sampled populations. (The distributions of the remaining two *bx bx* backcross groups were so similar to that of the original stock that they are omitted.) Symbols for the different groups are the same as in figure 1.

significantly different for the F_1 flies. However, for the *bx bx* stock there was a peak productivity on the second day. The difference between the peak day and the lowest (fourth) day, in percentage of total yield, was 11.7 per cent, and the day variance is significant at the .01 level. For the other populations the day effect was sometimes fairly significant also, but in any case no important influence was exerted on the main points of interest.

The mean daily yield for the wild stock, 68 eggs, is some 20 per cent lower than that reported by Bell *et al.* (1955) for non-inbred wild stocks. They state that nine such stocks, from various sources in the United States, showed no significant differences among themselves. Unless the stock used in the present experiment happens to be inferior, it seems probable that omission of acetic acid from the egg-collection caps is responsible for the difference. For the purposes of the experiment, however, the difference seems unimportant.

DISCUSSION

Five or six generations of backcrossing were evidently sufficient to cause a return of the *bx bx* production to the same level as that of the original stock, in spite of the enforced heterozygosity during the program. Evidently little or no effective selection for vigor had occurred in regard to the other chromosomes.

Since the *bx bx* F_2 yield was over four times that of the *bx bx* stock, it is clear that homozygosity for this gene is not the only basis for the poor performance of the stock. The role of *bx bx* is significant, however. If we compare the F_2 with the wild stock, reduction by a factor of .38 is evident. This factor may not be the true one since the F_2 performance may also have been influenced by other segregating factors, but it probably errs only in slightly exaggerating the role of the gene.

The effect of the "background" genotype is also shown by comparison of the normal *+/bx* backcross flies with the F_1, where *bx* is also heterozygous. The B6 yield is .29 of the F_1, and the yield of A5 has a factor of .37. The difference between B6 and A5 here possibly results from the additional generation of backcrossing for B6, but in view of the fact that B6 still has an entire chromosome III of wild origin, one might then have to assume that the "background" effect depended chiefly on material in chromosome II. A less plausible possibility is a disharmony between the intact wild III and the chromosomes from the *bx* stock. Still another possibility is a cytoplasmic or maternal influence. It is impossible to decide from our data between these alternatives.

Using .29 as the most probable reduction factor for "background," and .38 as the factor for *bx bx*, we should get a combined effect of .11 in the *bx bx* stock, if the factors operate independently. Compared with the wild stock, a mean yield of 30 eggs is calculated. This value is somewhat higher than actual; therefore, the probability of some interaction must be considered. If we calculate the reduction effect of *bx bx* in B6, comparing with *+/bx*, we obtain a factor of about .28. Similarly for A5, we get a factor of about .24. These values are both considerably different from the .38 obtained by calculating from F_2. This does indicate interaction effect with the different background.

Overdominance is not indicated at the *bx* locus, since the means of *+/bx* and *+/+* from A5 are essentially the same.

Robertson and Reeve (1955) have been especially concerned with variance effects in such studies. They note an inverse relationship between variance and heterozygosity, as revealed after conversion of the egg production data into natural logarithms. Conversion of our data has shown similar inverse relations for some of the groups, but not all. For example, the B6 *+/bx* showed less variance than F_1 in spite of the fact that the F_1 must be much more heterozygous. The coefficient of variation (table 1) shows a fairly consistent inverse relation to the mean. This seems to indicate a difference in precision of genetic control; that is, in the homozygotes with a small yield potential, the unit of measurement (one egg) is more subject to random error in production.

Hagberg (1953) argues that heterosis should be measured against the value of the superior parent instead of the mid-parent. A better standard, in our opinion, would be the species norm, so far as that is obtainable. For example, two very inferior inbred lines might produce a hybrid with normal yield; this would be a vast improvement over the parents but no advance in

relation to the species. Such an effect, interpreted by some as heterosis, would more logically be explained as concealment of different deleterious recessive "bottleneck" genes contributed by the two parents (Mangelsdorf, 1952).

The present F_1 population shows about a ten per cent improvement over the wild stock, which is presumed to agree with the species norm. Since the wild stock was not an inbred line, the cross is more comparable to a top-cross, but the improvement may be considered heterosis. Our data do not permit interpretation of the mechanism, but we can say that heterozygosity for the *bx* gene produced no reduction, at least.

Though our study shows that *bx* when homozygous acts as a "bottleneck" gene, it is a pertinent question how the stock acquired the additional inferior "background." We are unable to say whether this background consists of only one or many genes, but the evidence indicates that much or most of it is not linked with *bx*. The simplest interpretation is that in spite of supposed random mating as a result of mass transfer in maintenance of the stock, so few flies in each generation actually functioned as parents that there was in effect considerable inbreeding. Inbreeding depression by fixation of one or more deleterious genes present as "mutational load" (Muller, 1956) in the stock could then be the consequence. The failure to achieve recovery by reverse or other mutation over the considerably more than 100 generations of maintenance conforms to experience with inbred stocks of other species.

SUMMARY

Homozygous bx^{34e}, maintained over 100 generations as a closed stock, had an egg yield one-twelfth that of a random-bred wild stock. F_1 hybrids yielded ten per cent more eggs than the wild parental type. F_2 *bx bx* averaged over four times as productive as the original *bx* stock. Backcrossing F_1 at least five generations to the *bx* stock, always breeding from heterozygotes, resulted in a return of *bx bx* production to the *bx* stock level, while heterozygotes remained on a level intermediate between the parental stocks. Homozygous non-bithorax flies from the backcrosses were not different from heterozygotes, so that overdominance at this locus is not the probable cause of the F_1 heterosis. These results are interpreted to indicate that homozygosis for *bx* is about equal, in reducing-effect on yield, to that for "background" factors, and that there is probably some interaction of these in combination. It is suggested that the origin of the background factors in the *bx* stock may have been by random fixation of mutational load.

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Vol. XCIV, No. 879 The American Naturalist November–December, 1960

THE GENETIC LOAD DUE TO MOTHER-CHILD INCOMPATIBILITY*

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The concept of a genetic load has been useful in the analysis of animal and human populations (Morton, Crow, and Muller, 1956; Dobzhansky, 1957; Crow, 1958; and Morton, 1960). The genetic load is defined as the amount by which the population average fitness is impaired, or the incidence of specific types of morbidity, mortality, or infertility is increased, by the fact that the population is not of the optimum genotypic composition. The optimum genotypic composition need not, of course, be a single best genotype; the optimum population may have a variety of genotypes, depending, for example, on the amount of division of labor. The *mutation* load is due to recurrent harmful mutations, the *segregation* load is due to segregation of inferior homozygotes at loci where the heterozygote is favored, the *substitution* load (Haldane, 1957; Kimura, 1960) is due to the necessity for allele replacement in a changing environment, and there are others that can be defined and for which some possibility of measurement exists.

The *incompatibility load* comes not from any deficiency of a genotype itself, but from the fact that certain genotypes have a reduced fitness with certain parents. For example, an embryo of blood group A has a better chance of surviving if its mother is of group A than if she is group O. All known examples of the incompatibility load in the human population are due to serological differences, but future research may reveal other possibilities.

Consider first a locus with three alleles, of which the *ABO* system in man can serve as an example. Because of the rule that an individual can produce antibodies only against antigens he does not possess, and because an antigen is (with perhaps a few exceptions) the result of a dominant gene, it follows that any increased death rate will always be in heterozygotes.

We can summarize all potentially incompatible types by writing the maternal genotypes and the allele contributed to the embryo by the father. With random mating, they occur in the following proportions, letting p, q, and r stand for the frequencies of A, B, and O alleles.

Mother's genotype	Frequency (1)	Sperm genotype	Frequency (2)	(1)× (2)	Probability of death due to incompatibility
OO	r^2	A	p	pr^2	d_A
BB	q^2	A	p	pq^2	d_A
BO	$2qr$	A	p	$2pqr$	d_A
OO	r^2	B	q	qr^2	d_B
AA	p^2	B	q	qp^2	d_B
AO	$2pr$	B	q	$2pqr$	d_B

*Paper Number 783 from the Division of Genetics.

The total incompatibility load due to this locus is equal to

$$I = d_A(pr^2 + pq^2 + 2pqr) + d_B(qr^2 + qp^2 + 2pqr)$$
$$= d_A p(1-p)^2 + d_B q(1-q)^2 \quad (1)$$

The estimated death rate in incompatible embryos is .115 (Chung *et al.*, 1960). The total death rate from *AO* embryos with *O* mothers is therefore .013 as shown in table 1. The total proportion of deaths from the *A* antigen in all *A*-incompatible matings is .016. Assuming that the *B* antigen has the same effect, as the data of Chung *et al.* suggest, the total proportion of deaths due to incompatibility at the *ABO* locus is .024.

TABLE 1

Estimates of the genetic load due to the *ABO* blood group system. Item 7 is probably an underestimate, for reasons given in the text.

Cause of genetic load	Formula	Numerical value
1. Pre-natal deaths of *AO* children with *O* mothers	$d_{AO}pr^2$	.013
2. Pre-natal deaths due to *A* antigen	$d_A p(1-p)^2$	.016
3. Pre-natal deaths of all incompatible embryos	$d_A p(1-p)^2 + d_B q(1-q)^2$	.024
4. Pre-natal deaths of *OO* due to homozygote inviability	$s_{OO}r^2$	.034
5. Total pre-natal deaths due to homozygote inviability, assuming $s_{AA} = s_{BB} = s_{OO}$	$s_{OO}r^2 + s_{AA}p^2 + s_{BB}q^2$	.039
6. Total pre-natal deaths from incompatibility and homozygote inviability	3 + 5	.063
7. Total reduction in fitness at all ages from incompatibility and homozygote inviability	$s_{AO}p + s_{BO}q + s_{OO}r$	> .066

The following numerical values were used in the calculations: $p = .25$, $q = .08$, $r = .67$, $d_{AO} = d_{BO} = d_{BA} = .115$, and $s_{OO} = .076$.

More generally, if p_i represents the frequency of allele A_i and d_i is the decrease in fitness due to incompatibility for the antigen resulting from allele A_i, we can write the load as

$$I = \Sigma\, d_i p_i (1-p_i)^2 \quad (2)$$

where d_i may, of course, be zero for some alleles, as is probably the case for *O* in the *ABO* system.

This assumes that d_i is the same irrespective of the mother's genotype (assuming she has no A_i allele) and of the other (non A_i) allele in the child, which is a reasonable a priori assumption, but not necessarily true. If it is not, a separate d for each maternal-foetal genotype combination has to be introduced.

If either the mother or the child is inbred, the incompatibility load is changed (see Stern and Charles, 1945). Clearly the father's inbreeding coefficient is irrelevant.

1. *Mother inbred.* The load due to the antigen produced by allele A_i is now

$$d_i p_i[(1 - F_M)(\sum_{i \neq j} p_j)^2 + F_M \sum_{i \neq j} p_j]$$

$$= d_i p_i[(1 - p_i)^2(1 - F_M) + F_M(1 - p_i)]$$

$$= d_i p_i(1 - p_i)(1 - p_i + p_i F_M)$$

where F_M is the inbreeding coefficient of the mother.

Summed over all alleles, this is

$$D = \sum d_i p_i(1 - p_i)(1 - p_i + p_i F_M) \tag{3}$$

Thus the incompatibility load increases in proportion to the inbreeding coefficient of the mother.

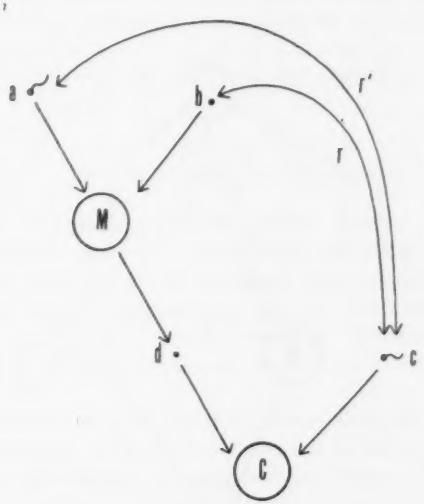

FIGURE 1. Path diagram for deriving the incompatibility load when the child is inbred. M = mother; C = child; a, b, c, and d are gametes.

2. *Child inbred.* The relations are shown in figure 1 where M is the mother and C is the child. The letters a and b are the two gametes that united to form the mother while c is the gamete contributed to the child by the father.

Let E be the probability that c is identical by descent with either a or b, in which case there can be no incompatibility. Then, letting $\equiv$ stand for identity by descent,

$$E = Prob\ (a \equiv c) + Prob\ (b \equiv c) - Prob\ (a \equiv b \equiv c)$$

$$= r + r' - Prob\ (a \equiv b \equiv c)$$

where r and r' are path coefficients from b to c and from a to c. But the inbreeding coefficient of the child, F_C, is $(r + r')/2$. Therefore

$$E = 2F_C - Prob\ (a \equiv b \equiv c) \tag{4}$$

where F_C is the inbreeding coefficient of the child.

The $Prob\ (a \equiv b \equiv c)$ is zero, unless there is a single ancestor from which all three gametes, a, b, and c, are descended. There seems to be no simple general rule for computing this, but it is easily done for individual cases. In most human pedigrees this term is zero.

The incompatibility load is then

$$I = D(1 - E)\,. \tag{5}$$

Hence, except for the possibility of a common ancestor of a, b, and c, the incompatibility load increases linearly with the inbreeding coefficient of the mother and decreases in proportion to the inbreeding coefficient of the child.

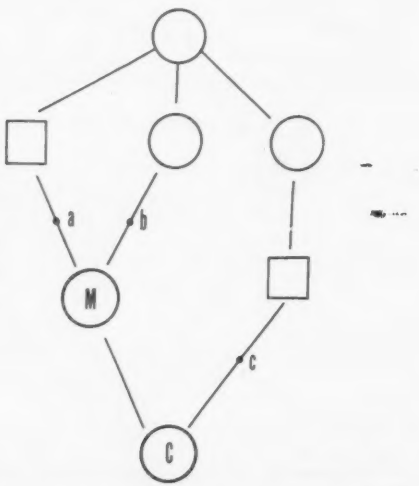

FIGURE 2. Hypothetical pedigree where gametes a, b, and c can all be traced to a common ancestor.

Figure 2 shows an example. This is a highly improbable pedigree for a human family, but it illustrates the difficulty when mother and child are both inbred because of the same common ancestor. The inbreeding coefficient of the child, F_C, is 1/16 by the usual methods. The probability that $a \equiv b \equiv c$ is 1/64. The inbreeding coefficient of the mother, F_m, is 1/8. $E = 2(1/16) - 1/64 = 7/64$.

Incompatibility does not lead to a stable polymorphism, but rather toward fixation of one of the alleles. This means that there must be some other

mechanism to maintain the polymorphism. Chung and Morton (1960) offer evidence that this is done in both the *ABO* and the *MN* systems by heterosis. This, too, causes a load in the population. It would appear that the total load of a locus must be considerably larger than, perhaps at least twice as large as, the incompatibility load in order that the polymorphism persist.

An estimate of the total load for the *ABO* locus can be gotten as follows. The formula for the increment of gene frequency change in one generation of selection is given by Wright (1959 and earlier) as

$$\Delta p_i = \frac{p_i(w_i - \bar{w})}{\bar{w}} = -\frac{p_i(s_i - \bar{s})}{\bar{w}} \tag{6}$$

where p_i is the frequency of the i^{th} allele, w_i is the average fitness in all its genotypic combinations of A_i, and $\bar{w}$ is the average fitness of the population. If the fitness is measured as a deviation, s, from an optimum genotype with fitness 1, then $s_i = 1 - w_i$, $\bar{s} = 1 - \bar{w}$, and $\bar{s}$ is the genetic load.

In any equilibrium population $\Delta p_i = 0$, and therefore $s_i = \bar{s}$. But, in a randomly mating population

$$\bar{s} = s_i = \frac{\sum_j p_i p_j s_{ij}}{p_i} = \sum_j p_j s_{ij} \tag{7}$$

where s_{ij} is the deviation from the optimum fitness of genotype A_iA_j. Therefore the genetic load for all the alleles at a locus may be estimated by choosing any particular allele, A_i, and, for every genotype into which this allele enters, multiplying the reduction in fitness by the frequency of the other allele; these products are then summed. This remarkable principle makes possible an estimate of the total load from knowledge of a single allele (Morton, 1960).

For the *ABO* system there is more information about the *O* allele than the others, so we choose it. The genetic load, due to both incompatibility and the polymorphism-maintaining selective factors, is by equation (7)

$$p s_{AO} + q s_{BO} + r s_{OO}. \tag{8}$$

The increased death rate of *AO* children, d_{AO}, can be estimated from existing data. The value of s_{AO}, in so far as this is determined by incompatibility, is given by multiplying d_{AO} by the fraction of all *AO* genotypes that come from incompatible matings. Thus $s_{AO} = d_{AO}p(r^2 + qr)/2pr = d_{AO}(1 - p)/2$. Similarly, $s_{BO} = d_{BO}(1 - q)/2$.

From the estimates of Chung, Matsunaga, and Morton (1960), $d_{AO} = d_{BO} = d_{AB} = .115$ (from incompatibility effects in heterozygotes) and $s_{OO} = .076$ (from decreased fitness in homozygotes). Taking the allele frequencies of *A*, *B*, and *O* to be .25, .08, and .67, the genetic load is estimated as .066 (table 1). With the Japanese frequencies, .27, .18, and .55, it is .062.

These are probably underestimates for two reasons: (1) the possibility that all heterozygotes are not of equal viability, apart from incompatibility

effects, is not considered in the calculations; (2) only pre-natal data were used in making the calculations; to the extent that the *OO* homozygotes have reduced viability in later life, the estimate should be increased.

The estimate of the genetic load, made in this way, includes selection at all ages for all alleles, including fertility differences. Yet, if *AA* and *BB* homozygotes have embryonic death rates as high as or higher than *OO*, much of the selection is in the embryonic stages, as shown in line 6 of table 1. This suggests that the bulk of the *ABO* selection is in the embryonic stages, rather than through association with adult diseases (see Chung and Morton, 1960).

Very little is known from direct observation about the fitness of *AA* and *BB* homozygotes. Since the locus appears to be in stable polymorphism, probably maintained by the heterozygotes having sufficient viability to offset incompatibility effects, and considering the equilibrium frequencies actually observed in human populations, the fitness of *AA* and *BB* are likely to be still lower than *OO*.

In any event, it is clear that the total effect of the *ABO* locus is very large. Since the total embryonic deaths are thought to be only about 30-40 per cent, this one locus contributes a substantial part. Clearly there cannot be many loci with such large contributions to the genetic load.

The high rate of increase with inbreeding of human childhood mortality and of certain diseases (Morton, Crow, and Muller, 1956; Morton, 1960) has been used as an argument that the genes concerned are maintained by recurrent mutation rather than by selective balances. Since the incompatibility load decreases rather than increases with inbreeding of the child, this also rules out incompatibility effects as a major contributor to such a load.

Detailed applications of the principles of this paper and a discussion of further implications are given by Chung and Morton (1960) and Chung, Matsunaga, and Morton (1960).

SUMMARY

The incompatibility load is defined as the decrease in fitness due to mother-child incompatibilities. Formulae for computing this load are given, and it is shown that the load increases linearly with the inbreeding coefficient of the mother and decreases in proportion to the inbreeding coefficient of the child. In a population with Caucasian allele frequencies, the amount of pre-natal death due to incompatibility at the *ABO* locus is estimated as .024. The expressed genetic load at this locus, due both to incompatibility and the polymorphism-maintaining selective factors, is estimated to be at least .066.

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Vol. XCIV, No. 879 The American Naturalist November–December, 1960

COMMUNITY STRUCTURE, POPULATION CONTROL, AND COMPETITION

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The methods whereby natural populations are limited in size have been debated with vigor during three decades, particularly during the last few years (see papers by Nicholson, Birch, Andrewartha, Milne, Reynoldson, and Hutchinson, and ensuing discussions in the Cold Spring Harbor Symposium, 1957). Few ecologists will deny the importance of the subject, since the method of regulation of populations must be known before we can understand nature and predict its behavior. Although discussion of the subject has usually been confined to single species populations, it is equally important in situations where two or more species are involved.

The purpose of this note is to demonstrate a pattern of population control in many communities which derives easily from a series of general, widely accepted observations. The logic used is not easily refuted. Furthermore, the pattern reconciles conflicting interpretations by showing that populations in different trophic levels are expected to differ in their methods of control.

Our first observation is that the accumulation of fossil fuels occurs at a rate that is negligible when compared with the rate of energy fixation through photosynthesis in the biosphere. Apparent exceptions to this observation, such as bogs and ponds, are successional stages, in which the failure of decomposition hastens the termination of the stage. The rate of accumulation when compared with that of photosynthesis has also been shown to be negligible over geologic time (Hutchinson, 1948).

If virtually all of the energy fixed in photosynthesis does indeed flow through the biosphere, it must follow that all organisms taken together are limited by the amount of energy fixed. In particular, the decomposers as a group must be food-limited, since by definition they comprise the trophic level which degrades organic debris. There is no a priori reason why predators, behavior, physiological changes induced by high densities, etc., could not limit decomposer populations. In fact, some decomposer populations may be limited in such ways. If so, however, others must consume the "left-over" food, so that the group as a whole remains food-limited; otherwise fossil fuel would accumulate rapidly.

Any population which is not resource-limited must, of course, be limited to a level *below* that set by its resources.

Our next three observations are interrelated. They apply primarily to terrestrial communities. The first of these is that cases of obvious depletion of green plants by herbivores are exceptions to the general picture, in which

the plants are abundant and largely intact. Moreover, cases of obvious mass destruction by meteorological catastrophes are exceptional in most areas. Taken together, these two observations mean that producers are neither herbivore-limited nor catastrophe-limited, and must therefore be limited by their own exhaustion of a resource. In many areas, the limiting resource is obviously light, but in arid regions water may be the critical factor, and there are spectacular cases of limitation through the exhaustion of a critical mineral. The final observation in this group is that there are temporary exceptions to the general lack of depletion of green plants by herbivores. This occurs when herbivores are protected either by man or natural events, and it indicates that the herbivores are able to deplete the vegetation whenever they become numerous enough, as in the cases of the Kaibab deer herd, rodent plagues, and many insect outbreaks. It therefore follows that the usual condition is for populations of herbivores *not* to be limited by their food supply.

The vagaries of weather have been suggested as an adequate method of control for herbivore populations. The best factual clues related to this argument are to be found in the analysis of the exceptional cases where terrestrial herbivores have become numerous enough to deplete the vegetation. This often occurs with introduced rather than native species. It is most difficult to suppose that a species had been unable to adapt so as to escape control by the weather to which it was exposed, and at the same time by sheer chance to be able to escape this control from weather to which it had not been previously exposed. This assumption is especially difficult when mutual invasions by different herbivores between two countries may in both cases result in pests. Even more difficult to accept, however, is the implication regarding the native herbivores. The assumption that the hundreds or thousands of species native to a forest have failed to escape from control by the weather despite long exposure and much selection, when an invader is able to defoliate without this past history, implies that "pre-adaptation" is more likely than ordinary adaptation. This we cannot accept.

The remaining general method of herbivore control is predation (in its broadest sense, including parasitism, etc.). It is important to note that this hypothesis is not denied by the presence of introduced pests, since it is necessary only to suppose that either their natural predators have been left behind, or that while the herbivore is able to exist in the new climate, its enemies are not. There are, furthermore, numerous examples of the direct effect of predator removal. The history of the Kaibab deer is the best known example, although deer across the northern portions of the country are in repeated danger of winter starvation as a result of protection and predator removal. Several rodent plagues have been attributed to the local destruction of predators. More recently, the extensive spraying of forests to kill caterpillars has resulted in outbreaks of scale insects. The latter are protected from the spray, while their beetle predators and other insect enemies are not.

Thus, although rigorous proof that herbivores are generally controlled by predation is lacking, supporting evidence is available, and the alternate hypothesis of control by weather leads to false or untenable implications.

The foregoing conclusion has an important implication in the mechanism of control of the predator populations. The predators and parasites, in controlling the populations of herbivores, must thereby limit their own resources, and as a group they must be food-limited. Although the populations of some carnivores are obviously limited by territoriality, this kind of internal check cannot operate for all carnivores taken together. If it did, the herbivores would normally expand to the point of depletion of the vegetation, as they do in the absence of their normal predators and parasites.

There thus exists either direct proof or a great preponderance of factual evidence that in terrestrial communities decomposers, producers, and predators, as whole trophic levels, are resource-limited in the classical density-dependent fashion. Each of these three can and does expand toward the limit of the appropriate resource. We may now examine the reasons why this is a frequent situation in nature.

Whatever the resource for which a set of terrestrial plant species compete, the competition ultimately expresses itself as competition for space. A community in which this space is frequently emptied through depletion by herbivores would run the continual risk of replacement by another assemblage of species in which the herbivores are held down in numbers by predation below the level at which they damage the vegetation. That space once held by a group of terrestrial plant species is not readily given up is shown by the cases where relict stands exist under climates no longer suitable for their return following deliberate or accidental destruction. Hence, the community in which herbivores are held down in numbers, and in which the producers are resource-limited will be the most persistent. The development of this pattern is less likely where high producer mortalities are inevitable. In lakes, for example, algal populations are prone to crash whether grazed or not. In the same environment, grazing depletion is much more common than in communities where the major producers are rooted plants.

A second general conclusion follows from the resource limitation of the species of three trophic levels. This conclusion is that if more than one species exists in one of these levels, they may avoid competition only if each species is limited by factors completely unutilized by any of the other species. It is a fact, of course, that many species occupy each level in most communities. It is also a fact that they are not sufficiently segregated in their needs to escape competition. Although isolated cases of non-overlap have been described, this has never been observed for an entire assemblage. Therefore, interspecific competition for resources exists among producers, among carnivores, and among decomposers.

It is satisfying to note the number of observations that fall into line with the foregoing deductions. Interspecific competition is a powerful selective force, and we should expect to find evidence of its operation. Moreover, the evidence should be most conclusive in trophic levels where it is neces-

sarily present. Among decomposers we find the most obvious specific mechanisms for reducing populations of competitors. The abundance of antibiotic substances attests to the frequency with which these mechanisms have been developed in the trophic level in which interspecific competition is inevitable. The producer species are the next most likely to reveal evidence of competition, and here we find such phenomena as crowding, shading, and vegetational zonation.

Among the carnivores, however, obvious adaptations for interspecific competition are less common. Active competition in the form of mutual habitat-exclusion has been noted in the cases of flatworms (Beauchamp and Ullyott, 1932) and salamanders (Hairston, 1951). The commonest situation takes the form of niche diversification as the result of interspecific competition. This has been noted in birds (Lack, 1945; MacArthur, 1958), salamanders (Hairston, 1949), and other groups of carnivores. Quite likely, host specificity in parasites and parasitoid insects is at least partly due to the influence of interspecific competition.

Of equal significance is the frequent occurrence among herbivores of apparent exceptions to the influence of density-dependent factors. The grasshoppers described by Birch (1957) and the thrips described by Davidson and Andrewartha (1948) are well known examples. Moreover, it is among herbivores that we find cited examples of coexistence without evidence of competition for resources, such as the leafhoppers reported by Ross (1957), and the psocids described by Broadhead (1958). It should be pointed out that in these latter cases coexistence applies primarily to an identity of food and place, and other aspects of the niches of these organisms are not known to be identical.

SUMMARY

In summary, then, our general conclusions are: (1) Populations of producers, carnivores, and decomposers are limited by their respective resources in the classical density-dependent fashion. (2) Interspecific competition must necessarily exist among the members of each of these three trophic levels. (3) Herbivores are seldom food-limited, appear most often to be predator-limited, and therefore are not likely to compete for common resources.

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Vol. XCIV, No. 879 The American Naturalist November-December, 1960

AN IMMUNOBIOLOGICAL MODEL OF IMPLANT-INDUCED URODELE SUPERNUMERARY LIMB FORMATION

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Nassonov (1935, 1936, 1938) has reported extensively on a series of studies which tested the ability of a variety of homoplastic and heteroplastic implants to induce the formation of accessory limb structures when lodged subcutaneously within the Axolotl limb field. These experiments utilized donor material of cartilage, lung, intestine, kidney, gill, liver, bladder, eyes, fins and gonads.

The present author (Ruben, 1955, 1957a, 1958) has previously reported similar results using frog kidney adenocarcinoma and normal frog kidney as donor implants in adult Triturus viridescens and larval Amblystoma hosts. These implants were lodged subcutaneously on the dorsal surface of the radio-ulnar portion of the forelimb. The results of experiments utilizing frog limb cartilage, skin, nerve, and muscle in adult Triturus and larval Amblystoma fore and hind limbs will be reported elsewhere.

Thus far, frog kidney seems to have been the most effective implant material tested with respect to percentage of induction and the quality of the morphogenetic result. Donor implants of equal volume from different organs, then, do not activate a new partial limb field with equal effectiveness. How can one account for these differences? Foreign donor material will cause a host to react against it via what is generally referred to as a homograft or heterograft response (for recent reviews see Lawrence, 1959; Merrill, 1959). This foreign body response will bring about the destruction of the implant material. Further, it is generally conceded that implant breakdown plays a role in initiating implant-induced accessory limb formation. It might be argued that qualitative differences in the products released by the donor material are responsible for the differing abilities of these organ implants to perform the task. However, it may also be argued that these differences are due to quantitative variation in the trauma initiated by the individual donor sources, such that the rate of degeneration of the implant would reflect the foreign body response of the host against it, and this in turn would determine (A) the quantity of breakdown products available to neighboring host tissues per unit time, and (B) the duration that sufficient quantities of these materials would be available to the host tissues. The foreign body response itself may vary with the character of the antigenic implant introduced, for example, its cellularity. Since genetic disparity is another factor which plays a role in determining this response, it might be expected that xenografts would be more effective than homografts in bringing about the necessary traumatic situ-

ation. In addition to this, normal frog kidney should be more effective than cancerous frog kidney since frog adenocarcinoma tubules "take" in salamander host limbs. The above suggestions are upheld by the results of experiments already cited. It would seem to me that the most likely explanation would represent a combination of the above alternatives; that is, cytolytic products from a variety of organ implants would undoubtedly differ and their quantity of release per unit time would in turn depend on the efficacy of the implant to initiate a foreign body response on the part of the host.

That the trauma brought about by implant-induced homograft or heterograft reactions may be merely one of many non-specific traumatic mechanisms capable of initiating urodele supernumerary limb formation is suggested by the experiments of Butler and Blum (1955) with ultra-violet light, Brunst (1959) with x-ray on tails, Della Valle (as cited in Needham, 1952) and Nassonov (cited in Brunst, 1959) with incisions and constrictions, and Breedis (1950) with carcinogens.

According to the quantitative thesis then, if any implant were to be destroyed too rapidly, so that the traumatic effect of its presence became diminished before neural and epithelial stimulation, and connective tissue and muscle dissociation had led to blastemal accumulation (this would be tantamount to early sloughing), then no accessory structures would be induced. In other words, the rate of implant breakdown may be improperly timed with respect to the responding morphogenetic processes for optimum results. This would apply to implant breakdown which is too slow as well. This being the case, implant dosage becomes an important factor to consider. It is equally plausible that excessive trauma might be just as ineffective as too low a traumatic level, since excessive trauma might interfere mechanically and physiologically with the morphogenetic processes of the limb. Certain sources of trauma production might even interfere with some specific component of the responding system. It may be that certain agents will initiate damage to the neural elements, for example, while others which bring about an equal amount of trauma do not. In addition to these complications it will be pointed out in further discussion that the experimental results indicate that in implant-induction systems, other factors may become superimposed upon the constitutional determinants of the heterograft or homograft response.

The model for viewing this phenomenon might be stated as follows: (1) The antigenic implant calls forth a foreign body response on the part of the host which proceeds to destroy the implant. (2) The cytolytic products of implant destruction can be pictured as acting directly on the neighboring peripheral nerve supply of the host limb. That the nerves play a major role in dissociating the limb structures, for example, muscle and connective tissue, in the region of the implant is suggested by the previously cited experiments which demonstrate that only a limited dissociation of the dermis overlying the implant is obtained in the absence of peripheral innervation. Dermal dissociation appears to be necessary in order for morpho-

genesis to proceed to a successful end result, in that it allows for the epidermal-deep mesodermal intimacy required for limb regenerating systems. Experiments by Bodemer (1959) which will be discussed in greater detail shortly, lead him to the same conclusion with respect to neural participation. (3) Once initiation of accessory regeneration is brought about, the implant-induced trauma must continue to act upon the neural elements for at least two weeks in larval host limbs and most likely for a longer period of time in adult host limbs. The processes of aggregation, subsequent growth, differentiation and morphogenesis of the blastema can be thought of as occuring in accordance with principles uncovered by investigations on amphibian limb regeneration in response to amputation. Delayed denervation experiments with implant-induced systems (Ruben, 1959) have pointed up this sequence of events. These results suggest that it may be possible that after dissociation takes place we are dealing with two neural thresholds, (a) an aggregation threshold which is sufficient to prevent dispersal of the accumulation blastema and which allows differentiation to proceed toward small cartilage nodules and (b) a growth threshold, a higher threshold, which stimulates the appearance of the usual high mitotic rate found in successful blastemata, successful in the sense that they yield a reasonably complete end product. The high growth rate would remove the possibility of precocious cartilaginous nodule differentiation from the blastema.

I wish now to consider how these ideas may relate to the recent experiments of Bodemer (1959). Although Bodemer's experimental systems differ significantly from those which led to this report, particularly with respect to the use of small homografts in association with deviated nerves, it is tempting to try to draw our results together on common ground. That Bodemer concluded from his work that "there does not appear to be a constant relationship between the rate of degeneration of implant tissues and their effectiveness in inducing supernumerary growth," may be a reflection of the experimental system he used. Bodemer's data correlate reasonably well with the cellularity of the donor organs, with three notable exceptions: (1) Bone and cartilage possess only moderate cellularity. However, their percentage of success in induction was relatively high. Evidence taken from celloidin (Ruben, 1957b) and frog cartilage experiments (Ruben, unpublished) indicates that hard irregularly shaped implants are traumatizing, perhaps by virtue of mechanical irritation, a factor which could override the homograft response in Bodemer's work. Actually one might expect that the matrix of the implant would protect the host by enclosing the cellular antigenic portions of the implant. The slow breakdown rate of the cartilage implants is indicative that this may have occurred, for in my own work, after 82 days, frog cartilage looks relatively healthy and induction is associated with these areas. Therefore, one is entitled to look for an extra-antigenic source for the trauma. The trauma associated with large frog bone implants was so extensive even after 82 days that it seemed to be playing a role in preventing accessory limb formation. The bony portions of the implants were always somewhat splintered, often projecting through

the skin cover and hence more irritating than cartilage to the neighboring host tissues. The smaller *homograft* bone fragments may have initiated a much lower level of disturbance, but one which was still sufficient to add to the dissociation stimulus coming from the nerve deviated along with the fragments. (2) Though Bodemer's spleen implants were highly cellular, they failed to induce accessory growths. This result might be explicable on the basis of considering that amphibian spleen is quite likely capable of forming a graft against host reaction (DeLanney, 1958). The composite of a graft against host reaction and host against graft reaction might interfere mechanically and physiologically with the establishment of a blastema. This argument might also be satisfactory as an explanation for the low percentage of induction with small intestine, since large lymphocytic populations are often found in the submucosa of this organ. (3) Kidney homograft failure represents the enigma in Bodemer's and Nassonov's work. It is difficult to account for the low percentage of support by urodele kidney on the basis of cellularity leading to a homograft response, particularly in the light of the excellent success obtained with frog kidney implants. At any rate, it is probably true that with the deviated nerve system, the homograft reaction may be of less import than if no extra nerve was supplied to the implant area. Certainly Bodemer's work points out that the qualitative completeness of the accessory limbs he gets is a function of the large amount of nerve he brings along. Only in urodele larvae will normal adult frog kidney xenografts alone yield such consistently grand supernumerary limbs.

A word or two might be said about the activity of muscle implants since both frog and salamander skeletal muscle implants disappear rapidly and prove to be weak inductors at best. It seems likely that cellularity in leading to a foreign body response is not playing a major role here, but that the relative ease of muscle dissociation may be of import; this is another possible extra-homo- or heterograft factor in this very complex post-embryonic system. This could account for the difference between skeletal and cardiac muscle in the homograft experiments as well, since cardiac muscle would be less likely to dissociate readily and it proves to be a better "inductor."

Bodemer also found that boiling destroyed the ability of the implant to stimulate accessory limb formation. This result could be explained on the basis of the removal of the cellular antigenicity of the implant thereby eliminating the homograft response. Hildemann (1958) has recently demonstrated that heat-killed scale grafts fail to elicit the homograft response in goldfish.

One must surely agree with Bodemer that alternative hypotheses exist and that "it is of considerable theoretical and practical importance to identify these factors which apparently fulfill a critical function at the outset of the regeneration process." Fortunately many of the suggestions offered here are subject to test, and experiments are under way which are designed to identify whether they are valid or not. I think that one would have to agree that homograft and heterograft responses must play a role in implant-induced accessory limb formation, but whether they are responsible for the

differing abilities of various organs to bring this about remains to be seen. Investigators should consider those factors which play a role in initiating a foreign body response when dealing with this post-embryonic "induction" phenomenon. It is at the very least a complicating factor.

SUMMARY

The possible relationship of homograft and heterograft reactions to stimulation of accessory urodele limb formation was discussed and a model, based upon the concept that implants taken from different organs may differ in their efficacy in initiating a host foreign body response and as such could theoretically deliver quantitatively varied amounts of qualitatively varied cytolytic products to neighboring host limb tissues, was proposed. Experiments, whose results seem to warrant consideration of the factors which play a role in the homograft and heterograft phenomena, were presented. It was suggested that though homograft and heterograft reactions play a role in this kind of post-embryonic "induction," by virtue of their relationship to implant-caused trauma, it is not yet known whether the varied "inductive" capabilities of different organs are directly related to them.

ACKNOWLEDGMENT

The investigations of the author were supported in part by a research grant (C-2913) from the Research Grants and Fellowship Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland. My appreciation goes to Dr. Charles Bodemer, who was kind enough to review the manuscript for me.

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ADDENDUM

Several papers and published discussions have appeared in the Fourth Tissue Homotransplantation Conference (Ann. of the N. Y. Acad. of Sci. 87: 1-607) subsequent to the time that the preceding paper was ready for publication, which provide experimental support for certain ideas and assumptions utilized to build the model. The keystone of the concept is the hypothesis that implants taken from different organs of the same donor will elicit varied homograft or heterograft reactions. The traditional view, stated by Scothorne and Nagy (pp. 144-155), is that "the homograft reaction, although individual-specific, is not tissue-specific, and an immune animal will react more quickly against any of the tissues of the original donor and not merely against the original immunizing tissue." Merrill (pp. 320-321) has, however, questioned this proposition, noting that testis and parathyroid act somewhat differently and further that one can produce immune sera against lymphocytes which will attack lymphocytes but not polymorphs. McKhann (pp. 321-322), considering the same issue, pointed out that his work with the weak H-3 locus in mice showed that different tissues did, in fact, act differently in producing second set skin reactions in response to pre-immunization. Bone marrow, buffy coat, and thymus were moderately effective. Renal epithelium and trypsinized epidermal cells were very effective, more effective than, or at least as effective as, primary set skin grafts; while spleen cells and lymph nodes were completely ineffective when placed intraperitoneally. He suggested that the antigens were not absent from the cells of less effective tissues, but that differences in ability to elicit homograft sensitivity by cells from a variety of sources, probably depends upon where the transplantation antigen(s) reside(s) within the cell. Albert (p. 322) agreed with this concept and pointed out that he has found that epidermal cells possess more violent antigenic properties than other living cells (for example, spleen or lymph node). Further, Amos (p. 276) in his considerations of the possible mechanisms of homograft destruction, also indicated that different organs stimulate different responses. He has cited literature which demonstrated that the predominant reactive host cell type against the donor material will vary with the different types of tissue transplanted.

Since the induction of homograft sensitivity seems to be restricted to nucleated cells, it may be reasonable then to consider that "the foreign body response itself will vary with the character of the antigenic implant introduced" and that its cellularity may play a quantitative role. That something akin to a homograft or heterograft response is, in fact, occurring in experiments which led to the preceding model is indicated by lymphocytic, fibroblastic, and polymorphonucleocytic infiltration of the implant sites with initial peripheral rather than central implant necrosis. Host attack on the implant appears to be the case, rather than a passive anoxic and non-nutritive disintegration which might be exhibited primarily in a central location within the implant mass.

That one is entitled to look for an extra-homo- or -heterograft factor in the case of cartilage implants is given weight by the report of Peacock, Weeks, and Petty (p. 175) who note that cartilage homografts persist by virtue of protection of the chondrocytes by the chondroitin sulfate-collagen matrix. Franklin (p. 183) has stated that chondroitin sulfate is non-antigenic and that collagen is a weak antigen at best.

It is to be noted that the immunobiological model which has been proposed is intended to apply only to *implant-induced* accessory growths. Other traumatic techniques will necessarily utilize different initial steps, though their post-initiation steps will presumably be the same.

Vol. XCIV, No. 879 The American Naturalist November–December, 1960

GENETIC AND NON-GENETIC FACTORS AFFECTING THE QUIVERING CONDITION IN MICE*

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Gruneberg (1952), in his second edition of *The Genetics of the Mouse*, lists 15 well established mutations with abnormal motion under the heading, "Central nervous system." Phenotypically these 15 neurological mutations may be classified into two large categories: waltzer-shaker type and trembler-waddler type. Ten of these 15 mutations belong to the former category and the remaining five belong to the latter. While a rapid, circular movement is a common characteristic of the waltzer-shaker type, this movement is absent in the trembler-waddler type. Instead, the latter shows various combinations of the following traits: (1) tremor, (2) paralysis of front or hind legs or of both, (3) muscular incoordination, (4) loss of straightening reflex, (5) locking hindlegs when picked up by the tail, (6) priapism, (7) epileptic form of convulsion, (8) reduction or loss of fertility.

Since Gruneberg's second edition, a large number of new neurological mutations, particularly of the trembler-waddler type, has been discovered and reported: (Haecker, Marinez, Markovic and Pizzaro, 1954; Phillips, 1954; Michelson, Russell and Herman, 1955; Lyon, 1955; Sirlin, 1956; Yoon and Les, 1957; Yoon, 1959). This large number of new mutations with similar phenotypic effects offers an excellent opportunity for the study of various problems in mouse genetics previously considered impossible or difficult. Accordingly, it was thought desirable to clarify the genetic as well as some non-genetic factors that may affect the expression of "neurological genes" as a starting point for further studies of these genes. Quivering (gene symbol, qv), a trembler-waddler type, is peculiarly suited to this purpose because of its diverse manifestations of the condition as previously reported (Yoon and Les, 1957). This is a simple recessive trait. Affected animals show various clinical symptoms in addition to their tremor. The disability is progressive, manifestation becoming more pronounced as the animals grow older. However, the degree and severity of affliction are not the same among these mice. They range from "rather mild" to "very severe" even among the sibs of the same age within an inbred strain. The purpose of this paper is to analyze the genetic and non-genetic factors which cause such wide variations in the degree and severity of the symptoms in quivering mice.

MATERIALS AND METHODS

The quivering strain (strain designation, Qv) is maintained by Qvqv × Qvqv sib matings. Animals were in the 11th generation of inbreeding at the start

*This work was supported by Grant No. B-1889 from the National Institutes of Health, Public Health Service.

TABLE 1

List of traits examined and grading system for them

Traits	Grading system	Description
Tremor	Graded from 0 through 7	Depending upon severity of tremor
Motility	" " " " "	Depending upon motile ability
Use of hindlegs	Graded from 0 through 3	Depending upon ability to use hindlegs during walk
Walking performance	" " " " "	Depending upon ability to walk on narrow bars without falling
Locking hindlegs	" " " " "	Depending upon frequency of locking and ability to relax after locking
Flexion of toes of hindlegs	" " " " "	Depending upon degree of flexion and ability to relax after flexion
Straightening reflex	" " " " "	Depending upon degree of ease with which animal can return to normal position when laid on its back

of the observation. A group of quivering mice from these matings is designated as Inbred. Some quivering females were outcrossed to Fs and BALB/c strains. Two groups of quivering mice recovered from these outcrosses were designated as Outcross 1 and Outcross 2 respectively. A total of 374 mice in three different populations was thus produced. Affected mice were weighed and examined at the third and the fourth week after birth. Thereafter they were weighed and examined once every two weeks until their natural death. Table 1 lists the various symptoms which were observed and numerically graded at each examination, together with the grading system.

FIGURE 1. Graph constructed from the distribution of deaths at various weeks of age in Inbred. The test of normality (for late death group) yields $g_1 = 0.186$ ($t = 0.789$, d.f. $= \infty$, $t_{0.05} = 1.96$) and $g_2 = -1.241$ ($t = 2.692$, d.f. $= \infty$, $t_{0.01} = 2.576$).

DESCRIPTION OF SYMPTOMS

A graph of the frequency distribution of deaths for Inbred is shown in figure 1. It is clearly bimodal with a large number of deaths taking place at the fourth and sixth week, reaching a low during the eighth through 12th week. Then there is a gradual climb to the second peak followed by a slow decline. It is evident that quivering mice have difficulty surviving the period immediately following weaning. Once past this critical period they live for a relatively long period of time for this type of mutant.

The degree of total affliction for any mouse may be scored as the sum of all the grades acquired for various traits observed. The possible maximum is 29. Mice of Inbred were divided into seven groups according to age at death and mean degrees at various ages were computed for each group. It is clear from the graphs as shown in figure 2 that the degree of total afflic-

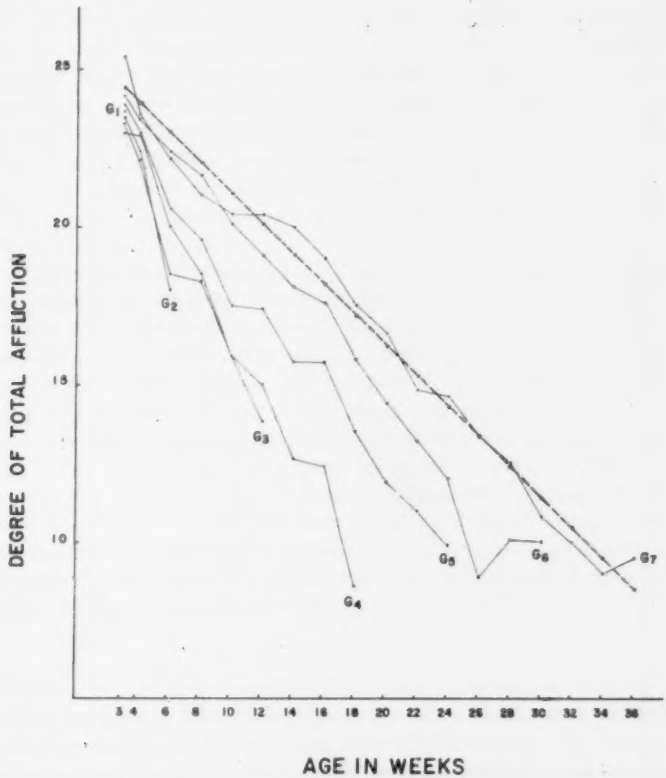

FIGURE 2. Degree of total affliction of quivering mice. Group 1 consists of mice found dead at fourth week; Group 2 at sixth and eighth; Group 3 at tenth, 12th and 14th; Group 4 at 16th, 18th and 20th; Group 5 at 22nd, 24th and 26th; Group 6 at 28th, 30th and 32nd; Group 7 at 34th, 36th and 38th week. G = group. The graph of broken line was constructed from intercepts for Group 7. Inbred.

tion falls linearly until the time of death. The difference between the regression coefficients of these two curves is statistically significant, indicating that those that die earlier show a more rapid deterioration.

There is a great variation in the degree of tremor among the affected mice. There is no difficulty in detecting the severely affected, but the mildly affected mice may, to the unaccustomed eye, look completely normal. The mean degree of tremor at various ages for Inbred is graphically shown in figure 3. It is rather slight at the third week but increases rapidly up to the eighth and through the 12th week and then gradually decreases as the animals grow older. This improvement during the later part of life is associated with a gradual loss of motility. When the data were fitted to a

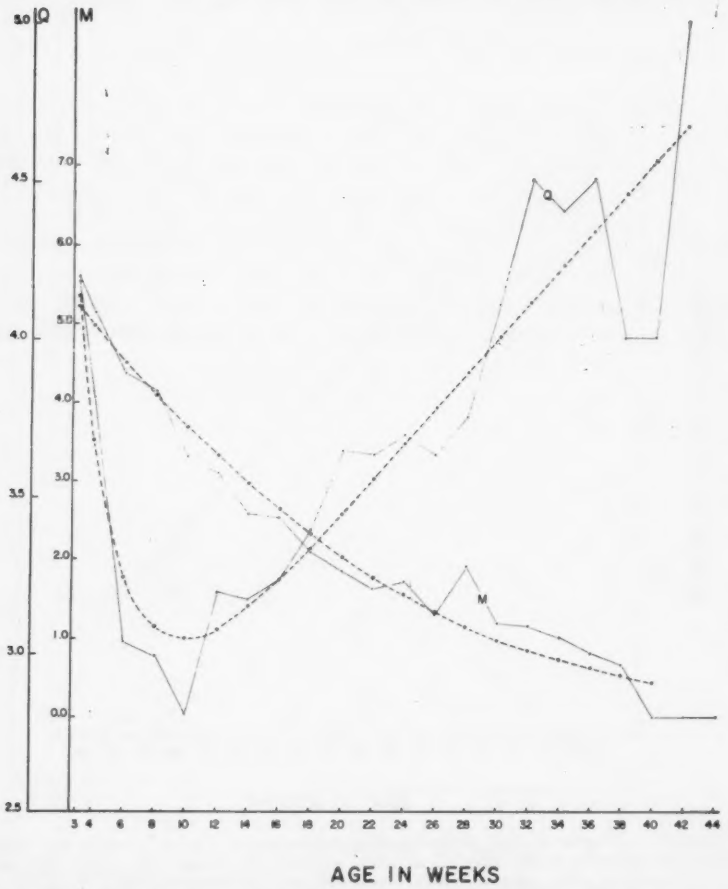

FIGURE 3. Degree of tremor and motility. Q for tremor; M for motility. Graphs of broken lines were constructed from intercepts. Inbred.

second degree polynomial after the ages of mice were transformed to a logarithmic scale, a satisfactory fit was obtained.

When affected animals are let loose on a flat surface they rarely move fast enough to escape from an observer. The motility is affected to a considerable extent as early as the third week and it worsens progressively, eventually reaching a stationary status. Figure 3 shows the mean degrees of motility at various ages, fitted to a curvilinear regression.

SEX

The effects of sex upon the quivering condition were studied by comparing males with females in all the traits observed. The results indicate a singular absence of differences between sexes.

GENIC BACKGROUND

All the preceding discussions were limited to the data from Inbred alone. Since these mice were produced in the inbred stock, it was assumed that their genic background was rather uniform and that the mice had attained a considerable degree of homozygosity through inbreeding. Mice in Outcrosses 1 and 2, however, were produced by outcrossing to two different strains. It is, therefore, assumed that Outcrosses 1 and 2 have higher heterozygosity and are different in their genic background from Inbred. Thus any difference observed among these three groups may be attributed to their genic background.

No difference which is statistically significant was observed between the three groups in the degree of total affliction. However, when the degree of tremor and of motility were compared, these three groups showed significant differences. The comparisons were made by graphs constructed for each group. In the degree of tremor the elevation of the curve for Outcross 2 is the highest, followed by Inbred and the elevation for Outcross 1 is the lowest. Various statistics show significant differences between these three curves. Mice in Outcross 1 are most severely and mice in Outcross 2 are least severely affected as far as tremor is concerned. In motility the positions of the two curves for Outcross 1 and Outcross 2 are reversed, indicating that mice in Outcross 2 are most severely and mice in Outcross 1 are least severely affected. It is clear that different genic backgrounds have differential effects upon different aspects of the condition.

SEASONAL CHANGES

Seasonal changes have considerable effects upon various aspects of the quivering condition. When the longevity of mice is arranged according to the dates of births, using the pooled data, the mean longevity was found to fluctuate smoothly with the seasons, being lower during cooler seasons and higher during warmer seasons.

Another seasonal effect was found in the incidence of priapism. Among a total of 188 males, 32 showed priapism. The incidence is considerably higher during cooler seasons and lower during warmer seasons, the difference being statistically significant.

AGE OF MOTHER

The effects of age of mother upon the longevity of their offspring were studied with the data from Inbred. It was found that quivering mice from older mothers tend to have shorter life spans. However, when the degree of tremor of mice from mothers of one age was compared with that of mice from mothers of another age, two groups at a time, none of the T values (Wilcoxan, 1945; White, 1952) was found to be significant. This is in agreement with the finding that no significant correlation exists between the degree of tremor and longevity.

SUMMARY

Quivering mice show various other symptoms in addition to tremor. The severity of these symptoms is not uniform. Genetic and non-genetic factors which cause such variations in the degree of various symptoms were studied.

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Vol. XCIV, No. 879 The American Naturalist November–December, 1960

LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

COMMENT ON DR. HUTCHINSON'S PAPER

One essential result in Hutchinson's paper (1960) is the fact that the D_{37} of ionizing radiations for either DNA or enzyme molecules has the order of magnitude of megarads. Since the observed D_{37} for cell inactivation, for instance in yeast, has the order of magnitude of kilorads, a large number (about 10^3) of specific indispensible molecules must be taken into account to be responsible for the observed cell inactivation. This is in good agreement with the results of an analysis of radiation inactivation of yeast cells investigated recently by means of certain experimental conditions and theoretical postulations (Stein and Laskowski, 1959; Laskowski and Stein, 1960).

This analysis revealed that x-ray inactivation of yeast cells was attributable mainly to recessive lethal mutations in a number of genes of the order of magnitude of 10^2. It could be calculated that a single gene needed about 0.7 megarad for a mutation-probability of 63 per cent. This is in fair agreement with 0.4 megarad for the γ-ray inactivation of the transforming ability of DNA in a wet cell in the presence of oxygen given in Hutchinson's paper. On the other hand, using radiation-induced complete budding inhibition as a criterion for enzyme inactivation, there was little or no indication of such an effect after x-ray doses resulting in about one per cent macroscopic survivals (that is, $\approx$ 150 kilorad for a diploid strain). An x-ray dose of 0.4 megarad was needed to inactivate about 50 per cent of diploid cells, presumably enzymatically according to the criterion mentioned above. Although several enzymes may be responsible for this specific effect of inactivation, their number was obviously too small to account for cell inactivation (that is, inhibition of formation of macroscopic colonies) in the kilorad range. With increasing ionizing density (LET), however, there was an increasing probability of enzyme inactivation in the cell. The latter was also found with increasing relatively low UV-doses. Disregarding the reduction by a factor ten of the roughly estimated number of molecular (genetic) units responsible for inactivation, the results cited by Hutchinson are in accordance with the genetical interpretation of radiation induced inactivation in yeast, which may be considered essentially as a destruction of macromolecules.

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REPLY BY DR. HUTCHINSON

The remarks by Drs. Stein and Laskowski on my paper are consistent, accepting their hypothesis that the killing of yeast cells is caused by recessive mutations. However, R. K. Mortimer, of the Donner Laboratory of the University of California, has shown most convincingly that a large share of the deaths is from dominant lethals. This work is ably reviewed by G. E. Magni, "Genetic Effects of Radiation on Yeast Cells and Genetic Control of Radiosensitivity," Radiation Research Suppl. 1: 347–356 (1959). With such definitive evidence for dominant lethals, I would be dubious of conclusions based on equations derived to treat the case of recessive mutations only.

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July 14, 1960

THE CONCEPT OF HETEROKARYOSIS IN THE FUNGI: THE HISTORICAL RECORD*

Although heterokaryosis is a well-established concept in mycology and has been a signal factor in unraveling the puzzle of variability in the fungi, an oversight concerning its origins has been rather prevalent in recent years. The purpose of this communication is to focus attention on this widespread misconception which is well illustrated by the following statement of W. C. Snyder in the recent volume, *Plant Pathology Problems and Progress*: "The phenomenon [heterokaryosis] in fungi was first demonstrated beyond question by Hansen and Smith in 1932 and it was they who first applied the term heterokaryosis in the fungi." This opinion is apparently shared by the majority of biologists presently interested in the subject of heterokaryosis in fungi (Beadle and Coonradt, 1944; Dodge, 1945; Pontecorvo, 1946; Raper, 1953), since only two statements contrary to this opinion have been found by the writer in the literature of the last three decades (Raper and San Antonio, 1954; Raper, in press). Here reference is made to the work of H. Burgeff (1914) on heterokaryosis in *Phycomyces nitens*.

In contrast to the recent literature this work of Burgeff was apparently given wide recognition prior to 1930 (Blakeslee, 1920; Brierley, 1920, 1922, 1929; Kniep, 1920, 1928; Gäumann, 1926). The work was presented in two principal papers (Burgeff, 1912, 1914) and was, primarily, a study of variation, sexuality and inheritance in the mucor *Phycomyces nitens*. In spite of the complexity of these problems (especially in 1912–14), Burgeff's handling of them makes the work a landmark in mycological research. In the two papers he introduced the concept of heterokaryosis in the fungi and defined the terms heterokaryotic and homokaryotic. He realized the relationship between the nuclear constitution of the sporangic spore and the phenotype of the resulting mycelium and developed the rationale for the production of three types of spores by a heterokaryotic mycelium (Hansen and Smith's two homotypes and one heterotype) on the basis of chance nuclear assortment and association during spore formation. He also realized that vegetative dissociation of a heterokaryon into its homokaryotic components would be an explanation of the sectoring which occurred in heterokaryotic cultures. Finally, he postulated that differential rates of division of the two nuclear types in a heterokaryon could be a factor in its gradual conversion into one of its homokaryotic components. His experiments on these various points are well designed, and the one technique which he devised to synthesize particular heterokaryons in this difficult material displays a marked ingenuity.

Clearly heterokaryosis in fungi, both the concept and the term, did not originate in 1932 but rather in 1912. It is hoped that this communication will help to dispel the present prevailing attitude and that Burgeff will once again be given the credit due him for his pioneering study of a most important aspect of experimental mycology.

*Supported by Public Health Service Grant No. E 2326.

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NEW YORK, NEW YORK
April 27, 1960

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CURRENT PERIODICAL SERIES

PUBLICATION NO.: 3

TITLE: AMERICAN NATURALIST

VOLUME: 95 ISSUES: 880-885

DATE: January - December, 1961